Glaucome embodies a group of disease processes associated with an increase in intraocular pressure (IOP) that results in retinal ganglion cell death, optic nerve axonal loss, and eventual blindness. Glaucoma is a major cause of irreversible blindness in dogs, with an incidence of 0.5%. Medical treatments of glaucoma, including topical administration of adrenergic agonists and prostaglandin analogs and oral or topical administration of carbonic anhydrase inhibitors, have been evaluated in dogs. Methazolamide is an orally administered carbonic anhydrase inhibitor that is used widely in the treatment of glaucoma in dogs.

Aqueous humor is made by 3 mechanisms that include diffusion, ultrafiltration, and active transport (which involves carbonic anhydrase). Carbonic anhydrase was identified in the ciliary process of the rabbit more than 50 years ago. Carbonic anhydrase is a catalyst of the reversible reaction of H₂O and CO₂ in equilibrium with H⁺ and HCO₃⁻. The bicarbonate ion is actively transported across the ciliary epithelium into the posterior chamber, with sodium the primary accompanying cation, thus establishing an osmotic gradient. Water follows the osmotic gradient, resulting in aqueous humor formation. The carbonic anhydrase inhibitor acetazolamide was subsequently shown to reduce IOP. Carbonic anhydrase inhibitors block the formation of the bicarbonate ion by the ciliary body, thus reducing IOP. Greater than 99% of the carbonic anhydrase activity must be inhibited before aqueous humor secretion is inhibited. A single oral dose of methazolamide at 5 mg/kg is reported to decrease IOP by 19.71% in normotensive Beagles and 28.04% in glaucomatous Beagles, with maximal effect at 3 hours and 6 hours, respectively. In the same study, an oral dose of methazolamide at 2.5 mg/kg resulted in a decrease in IOP of 10.87% and 20.50%, respectively. The AHFRs in both treatment groups were significantly lower than pretreatment AHFRs.

The purposes of the study reported here were to determine the magnitude and duration of the effect of oral administration of methazolamide at 2 dosages on IOP in dogs in single-dose and multiple-dose trials and to determine aqueous humor flow rate (AHFR) by use of anterior segment fluorophotometry before and during treatment.

Materials and Methods

Twenty-five adult Beagles weighing 12 to 16 kg were included in our study. All eyes were examined...
and determined to be free of ocular disease on the basis of results of slit-lamp biomicroscopy, Schirmer tear tests, gonioscopy, applanation tonometry, and binocular indirect ophthalmoscopy. Physical examination revealed that dogs were clinically normal. Dogs were housed in the same room under cyclic illumination (12 hours of light and 12 hours of darkness). Our study was conducted in accordance with the Animal Care and Use Committee guidelines at the University of Tennessee.

During a 1-week acclimatization period, IOPs were measured 3 times a day via applanation tonometry without topical anesthetic. The same investigator (BJS) performed all tonometry readings. The tonometer was calibrated at every time point prior to use. Three readings with < 5% variance were recorded and averaged for each eye at each time point. Aqueous humor flow rates were obtained by use of a computerized scanning ocular fluorophotometer as previously described.

Dogs were randomly placed in 2 treatment groups and a control group. To blind the principal investigator (BJS), each dog was fed a meatball of canned dog food at each treatment period. For dogs in the treatment groups, methazolamide tablets were buried in the meatball by 1 of the other investigators. Animals in the control group were fed a meatball without methazolamide. Baseline IOPs were measured at 8 and 10 AM and at 1, 6, and 9 PM on day 0. Baseline AHFRs were determined on day 1. On day 2, the single-dose trial was initiated with oral administration of 25 mg of methazolamide (mean dosage, 2.84 ± 0.48 mg/kg) in 10 dogs and 50 mg of methazolamide (mean dosage, 5.83 ± 0.80 mg/kg) in 10 dogs at 7 AM. Five dogs were in the control group. The dose was chosen on the basis of the findings in a prior study and recommendations.

On day 3, the multiple-dose trial was initiated. Group assignments for the single-dose trial were maintained in the multiple-dose trial. On days 3 through 9, the treatment or control meatballs were administered at 7 AM and at 3 and 11 PM. The frequency of dose administration was chosen on the basis of the results of a prior study and recommendations. Intraocular pressures were measured at 10 AM and at 1, 6, and 9 PM on days 3 through 12. On day 8, anterior segment fluorophotometry was repeated.

Statistical analysis—Mean IOP and AHFR of both eyes were used in all calculations. Baseline IOPs were compared across time by use of an ANOVA for repeated measures. Baseline IOPs were compared with IOPs after treatment by use of a paired t-test for each time point in the single-dose trial. In the multiple-dose trial, the mean IOPs for each dosage group at each time point during the 7-day treatment period (days 3 through 9) were used in all calculations. Baseline IOPs, treatment period IOPs, and post-treatment period IOPs (days 10, 11, and 12) for each time point were compared by use of a repeated measures ANOVA and the Bonferroni’s t-test with Sidak correction for multiple pair-wise comparisons. Baseline AHFRs were compared with treatment period AHFRs by use of paired t-tests. Values of P < 0.05 were considered significant.
Intraocular pressures varied diurnally with the highest IOPs in the morning (Fig 1). In the single-dose trial, IOPs of the 25-mg treatment group dogs were significantly decreased at 8 and 10 AM, compared with baseline values. Intraocular pressures of the 25-mg treatment group dogs were significantly increased at 6 PM, compared with baseline values (Fig 2). Intraocular pressures of the 50-mg treatment group dogs were significantly decreased at 8 and 10 AM and 1 PM, compared with baseline values. Intraocular pressures of the 50-mg treatment group dogs were significantly increased at 9 PM, compared with baseline values. The greatest decrease in IOP for both treatment groups was at 10 AM (18 and 21% for the 25-mg and 50-mg treatment groups, respectively).

In the multiple-dose trial (days 3 to 9), dogs in the 25-mg and 50-mg treatment groups had significantly lower IOPs during the treatment period at 10 AM and 1 PM, compared with baseline values, but not at 6 and 9 PM. Maximum decreases in IOP were 13 and 19% in the 25-mg and 50-mg treatment group dogs but not in the control group dogs. There was no significant difference in IOP between the 2 treatment groups. See Figure 2 for remainder of key.

Aqueous humor flow rates in the 25-mg treatment group (mean, 4.15 ± 1.18 µL/min) and the 50-mg treatment group (mean, 3.23 ± 1.06 µL/min) were significantly lower than the pretreatment AHFR (mean, 5.10 ± 1.98 µL/min), but there was no significant difference between the 2 treatment groups (Fig 4). The decrease in AHFR was correlated with the change in IOP during treatment (Fig 5).

Discussion
Numerous IOP-lowering medications have been evaluated in dogs. Methazolamide is an orally adminis-
tered carbonic anhydrase inhibitor that is widely used for the treatment of glaucoma because of its availability and low adverse effects, compared with other available carbonic anhydrase inhibitors. In a previous study, methazolamide was the most effective of 4 carbonic anhydrase inhibitors evaluated in normal and glaucomatous Beagles, lowering the IOP by a maximum of 28%. This was a single-dose trial in which maximal effects were recorded at 3 to 6 hours after treatment, and the duration of action was > 8 hours. Results of another study confirmed a significant decrease in IOP in glaucomatous Beagles following methazolamide treatment during a 5-day period.

In our study, assessment of the efficacy of methazolamide was made by measuring IOP via tonometry and by measuring AHFR via fluorophotometry. Although the instrument used for tonometry in our study has inherent variability in measurements, it provides a useful experimental modality that accurately and reliably measures IOP in dogs in the range of 0 to 30 mm Hg (as determined by manometry). Aqueous humor flow rates measured fluorophotometrically correlate well with known rates of flow in perfusion models, making fluorophotometry the gold standard of AHFR measurement. Therefore, these modalities allow measurements that can be analyzed to determine the efficacy of a drug.

In the single-dose trial of our study, both the 25- and 50-mg treatment group dogs had a decrease in IOP, compared with baseline values (maximum decrease of 18 and 21%, respectively). This effect lasted at least 3 hours in the 25-mg treatment group dogs and 6 hours in the 50-mg treatment group dogs. In both groups, the IOP increased significantly several hours after administration of the drug, compared with baseline values. This was a consistent finding occurring in 100% of dogs treated with methazolamide. This rebound in IOP following discontinuation of methazolamide administration was an interesting, although unanticipated, finding. Willis et al reported a similar effect in horses treated with once daily topical administration of a carbonic anhydrase inhibitor and speculated that the rebound was associated with discontinuation of the drug. One could speculate that suppression of carbonic anhydrase induces up-regulation of enzyme activities to maintain intraocular homeostasis. Although this phenomenon has not been investigated with respect to carbonic anhydrase, changes in other constitutive aqueous humor elements have been linked to methazolamide. Up-regulation of carbonic anhydrase would certainly explain a sudden increase in IOP following cessation of methazolamide, as the mechanism for an increase in aqueous production would be in place. This hypothesis might also explain the common clinical observation of refractoriness to oral administration of carbonic anhydrase inhibitors in some dogs on long-term treatment. It would not explain, however, why the same phenomenon was not observed by Cavarse et al in dogs following topical administration of the carbonic anhydrase inhibitor dorzolamide hydrochloride. We hypothesize that carbonic anhydrase inhibitors delivered by different routes affect membrane-bound versus cytosolic carbonic anhydrase differently. Although isoenzyme IV of carbonic anhydrase located on basolateral membranes of nonpigmented ciliary epithelial cells undoubtedly plays a critical role in IOP control, the function (and even the existence) of isoenzyme IV in the cytosol of nonpigmented ciliary epithelial cells is less clear.

In the multiple-dose trial of our study, a significant decrease in AHFR was observed in response to treatment. The IOP-lowering efficacy varied with time of day, with a significant decrease in IOPs at 10 AM and 1 PM but not at 6 and 9 PM during the treatment period. We speculate that diurnal variability in AHFR may be responsible for this phenomenon in that a constant fractional decrease in aqueous humor production induced by methazolamide would translate into a greater absolute decrease in aqueous humor production during times of maximal aqueous production (ie, in the morning). This greater absolute decrease would result in a more noticeable effect on IOP. Diurnal variation in IOP has been previously reported for dogs. As with previous reports, our dogs had higher IOPs in the morning. Diurnal variation in facility outflow in humans and fluctuations in aqueous humor production are likely factors responsible for diurnal variation in IOP. Demonstration of lower AHFRs in humans during sleep, compared with waking hours, corroborates this hypothesis. A similar correlation between diurnal variation in AHFRs and diurnal variation in IOP has also been observed in rabbits. We hypothesize that diurnal variability in AHFR may also explain diurnal variability in IOP in dogs, and hence the differential effects of methazolamide on IOPs at 10 AM and 1 PM versus 6 and 9 PM.

Fluorophotometric assessment of AHFR is based on serial measurements of corneal and aqueous humor concentrations for several hours following corneal fluorescein loading. Calculated AHFRs are therefore a reflection of aqueous dynamics during a wide range of time, beginning with corneal loading and ending with the final fluorescein measurement in the aqueous. In our study, we applied fluorescein at 7 AM and made the last fluorescein measurement at approximately 5 PM. Our measurements therefore reflect aqueous flow in the morning and early afternoon; our observed decrease in AHFR is consistent with the decrease in IOPs at 10 AM and 1 PM. To correlate 6 and 9 PM IOPs with the AHFR would require corneal fluorescein loading in the early afternoon and fluorescein measurements shortly after midnight, which was not done in our study.

In a previous study on clinically normal dogs, the topical administration of the carbonic anhydrase inhibitor dorzolamide hydrochloride did not result in variation of the IOP-lowering effect with regards to time of day, as was found in our study with oral administration of methazolamide. This may have occurred because dorzolamide has a greater inhibitory effect on carbonic anhydrase than methazolamide, resulting in greater suppression of aqueous humor formation. Concentrations of dorzolamide and methazolamide in the ciliary body are probably different as well, owing to different routes of administration, which would also explain the superior efficacy of dorzolamide in the evening despite diurnal variability in IOP.
The baseline AHFR (approx 5 µl/min) that was measured in our study is similar to previously reported values.\(^3,6,17\) In our study, oral administration of methazolamide resulted in a significant decrease in the AHFR, which indicates the effectiveness of the drug. There was, however, no significant difference in the AHFR between the 25-mg and 50-mg treatment groups of dogs, indicating no additional benefits to an increase in AHFR between the 25-mg and 50-mg treatment group. However, there was no significant difference in the values.\(^16,17\) In our study, oral administration of methazolamide resulted in a significant decrease in the plasma to posterior chamber: effect of acetazolamide and relation to the treatment of glaucoma.

References


