Effects of the topically applied calcium-channel blocker flunarizine on intraocular pressure in clinically normal dogs

Andrew L. Greller, VMD; Allison R. Hoffman, DVM; Chengcheng Liu, MS; Gui-shuang Ying, PhD; Daljit K. Vudathala, PhD; Gregory M. Acland, BVSc; András M. Komáromy, DrMedVet, PhD

Objective—To determine effects of the topically applied calcium-channel blocker flunarizine on intraocular pressure (IOP) in clinically normal dogs.

Animals—20 dogs.

Procedures—Baseline diurnal IOPs were determined by use of a rebound tonometer on 2 consecutive days. Subsequently, 1 randomly chosen eye of each dog was treated topically twice daily for 5 days with 0.5% flunarizine. During this treatment period, diurnal IOPs were measured. In addition, pupillary diameter and mean arterial blood pressure (MAP) were evaluated. Serum flunarizine concentrations were measured on treatment day 5. Intraday fluctuation of IOP was analyzed by use of an ANOVA for repeated measures and a trend test. Changes in IOP from baseline values were assessed and compared with IOPs for the days of treatment. Values were also compared between treated and untreated eyes.

Results—A significant intraday fluctuation in baseline IOP was detected, which was highest in the morning (mean ± SE, 15.8 ± 0.63 mm Hg) and lowest at night (12.9 ± 0.61 mm Hg). After 2 days of treatment, there was a significant decrease in IOP from baseline values in treated (0.93 ± 0.35 mm Hg) and untreated (0.95 ± 0.34 mm Hg) eyes. There was no significant treatment effect on pupillary diameter or MAP. Flunarizine was detected in serum samples of all dogs (mean ± SD, 3.89 ± 6.36 μg/L).

Conclusions and Clinical Relevance—Topically applied flunarizine decreased IOP in dogs after 2 days of twice-daily application. This calcium-channel blocker could be effective in the treatment of dogs with glaucoma. (*Am J Vet Res* 2008;69:273–278)

Glaucomatous optic neuropathy is a leading cause for blindness in humans[1] and dogs.[2] It consists of a group of neurodegenerative diseases characterized by the progressive loss of retinal ganglion cells and their axons.[3] The pathophysiologic process of glaucomatous optic neuropathy is not fully understood, but it is likely to be a multifactorial event. Increases in IOP are considered a major risk factor.[3] Separately or in addition to IOP, other factors (such as dysfunction of ocular blood-flow regulation with local ischemia-hypoxia,[4,5] excessive stimulation of the glutamatergic system,[6] and aberrations in immunity[7]) can contribute to death of retinal ganglion cells.

Abbreviations

IOP	Intraocular pressure
MAP	Mean arterial blood pressure

Currently, treatments for patients with glaucoma focus on decreasing IOP by medical and surgical methods.[8] In addition to standard medical agents, such as prostaglandin analogues, carbonic anhydrase inhibitors, α_2-receptor agonists, β blockers, and cholinergic agonists, use of calcium-channel blockers has also been proposed[9] for the treatment of patients with glaucoma. Calcium-channel blockers are not generally recommended as a therapeutic approach for humans with glaucoma because there is a lack of consensus regarding long-term clinical outcome from randomized placebo-controlled studies.

Ocular effects of various calcium-channel blockers have been studied in selected species, such as rats,[10,11] rabbits,[12-20] cats,[21] dogs,[22] monkeys,[12,23,a,b] and humans.[9,19,24] In addition to their direct neuroprotective effects on retinal ganglion cells,[11,25] calcium-channel

Received May 2, 2007.
Accepted July 5, 2007.
From the Eye Care for Animals, 2316 W Northern Ave, Phoenix, AZ 85021 (Greller); the Eye Care for Animals, 150 N San Gabriel, Pasadena, CA 91107 (Hoffman); the Department of Ophthalmology, School of Medicine (Liu, Ying), and Departments of Pathobiology (Vudathala) and Clinical Studies (Komáromy), School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA 19104; and the James A. Baker Institute for Animal Health, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853 (Acland). Dr. Greller's present address is the Eye Care for Animals, 6651-F Backlick Rd, Springfield, VA 22150.
Supported by the National Institutes of Health, National Eye Institute (EY006855, K12 EY015398, and P30 EY001583), and Foundation Fighting Blindness.
Presented in part at the American College of Veterinary Ophthalmologists Annual Meeting, San Antonio, Tex, November 2006.
The authors thank Amanda Nickle, Gerri Antonini, Alice Eidsen, and Tracy Greiner for technical assistance.
Address correspondence to Dr. Komáromy.

blockers can also increase ocular blood flow[12,20-22,25,26] and help to prevent retinal ischemia.[10,17] Calcium-channel blockers can increase[15] or decrease IOP.[16,18,23] In a number of reports,[14,17,23,a,b] investigators indicated that topical application of the calcium-channel blocker flunarizine, a difluorinated piperazine derivative, lowers the IOP in clinically normal monkeys and rabbits. The objective of the study reported here was to evaluate the effect of topically applied flunarizine on the IOP of clinically normal dogs.

Materials and Methods

Animals—Twenty healthy mixed-breed dogs (12 males and 8 females; mean ± SD age, 60.5 ± 25.6 months; mean body weight, 13.9 ± 3.3 kg) were used for the study. It was determined that these dogs had normal eyes on the basis of an ocular examination that included evaluation by use of slit-lamp biomicroscopy, binocular indirect ophthalmoscopy, Schirmer tear testing, fluorescein staining, and tonometry. All procedures were approved by the Institutional Animal Care and Use Committee of the University of Pennsylvania and were performed in accordance with the Association for Research in Vision and Ophthalmology statement for the Use of Animals in Ophthalmic and Vision Research.

Study design—Two consecutive days of baseline diurnal IOP measurements (baseline days 1 and 2) were used to familiarize the dogs with the techniques for data collection and to determine the typical diurnal IOP variations. This was followed by 5 days of topical treatment with flunarizine in 1 eye of each dog.

On each day of the study, tonometry was performed at the same 10 times (8, 8:30, 9, 9:30, and 10 AM and 12, 2, 4, 6, and 8 PM). The same investigator (ALG) performed all IOP measurements; a rebound tonometer[e] was used. The same instrument was used consistently throughout the baseline and treatment periods; it was calibrated and used in accordance with the manufacturer's recommendations. Dogs were gently and manually restrained without the use of sedatives; data collection was performed in the same quiet and familiar environment with the assistance of the same dog handlers. Topical anesthesia was not used. Because IOP can be affected by repeated pressure measurements, the first IOP reading (mean of 6 separate values) with < 5% error was used and recorded.

On the first treatment day, a coin toss was used to determine which eye of the first dog would receive flunarizine treatment. The contralateral eye served as the control eye. The treated and control eyes were then alternated in each subsequent dog so that an equal number of left and right eyes received flunarizine treatment.

Flunarizine dihydrochloride is a highly lipophilic drug and thus is difficult to dissolve in water. To limit variables associated with drug bioavailability, flunarizine[d] was dissolved in 50% polyethylene glycol (pH, 4.0), similar to the procedure used in other studies,[17,a,b] to obtain a 0.5% solution. The resulting solution had a pH of 1.8. Buffering of the solution to a pH > 2.1 resulted in drug precipitation. Therefore, despite the low pH, the unbuffered 0.5% flunarizine solution was used.

The application of l drop (50 µL) of 0.5% flunarizine immediately followed the IOP measurements obtained at 8 AM and 2 PM on each day of the 5-day treatment period. One drop of vehicle (50% polyethylene glycol) was administered to the contralateral control eye at the same time that the flunarizine was administered to the treated eye.

Measurement of other clinical variables—Pupillary diameter was measured along the horizontal pupillary axis of each eye. After a 2-minute adaptation period at preset ambient light conditions (0.001 mW/cm^2), a Jameson caliper was used to measure pupillary diameter. Measurements were obtained at 5 PM on baseline day 2 and also on day 5 of treatment.

The MAP was measured indirectly[e] in the right hind limb by the same investigator (ALG) and assistants at 4 PM on baseline day 2 and also on treatment day 5. Three sequential MAP measurements were obtained, and the mean value for the measurements was calculated and recorded for each dog on each of those 2 days.

A complete ophthalmic examination was performed within 1 hour after the 2 PM treatment was administered on days 1 and 5. A modification of the Hackett and MacDonald scoring system[27] was used to compare the ocular irritative response (ocular discharge, conjunctival hyperemia and swelling, corneal cloudiness and uptake of fluorescein stain, iris injection, and aqueous flare) between treatment and control eyes.

Determination of serum flunarizine concentrations—Blood samples were collected from each dog within 27 to 33 minutes after the 2 PM treatment was administered on the last treatment day. Samples were collected via jugular venipuncture and allowed to clot. Once clotted, samples were immediately centrifuged, and the serum was harvested and stored at –80°C until analysis.

Each serum sample was mixed with 2 volumes of acetonitrile and filtered through a 0.22-µm nylon filter[f] to remove protein precipitate. The clear filtrate was used for analysis and injected into a liquid chromatography–mass spectrometry system.[g] The concentration of drug in serum was measured by use of a calibration curve prepared by spiking control serum with known amounts of flunarizine.

Data analysis—Data analyses were performed by use of a software program. Significance was set at values of $P \leq 0.05$. Interday and intraday IOP fluctuations during the 2 baseline days were assessed by use of a repeated-measures ANOVA. Sources of variation considered in the model were time, day, time-by-day interaction, animal, and random measurement error. Time, day, and the time-by-day interaction were considered fixed effects. Random effects were attributed to variation for each animal and random error for each observation.

To evaluate the effect of topical application of flunarizine on IOP of treated and untreated eyes, we calculated the change in IOP from the value on baseline day 2, given that the difference in IOP between baseline days 1 and 2 could have resulted from the dogs' adjustment to the protocol. We tested whether the change in IOP from the value for baseline day 2 was significantly different from 0 on each treatment day for treated and untreated eyes. We also compared the change in IOP

between treated and untreated eyes and among days of treatment. These comparisons were made by use of generalized estimating equations that accounted for the correlations from repeated measures and correlations from paired eyes, which were executed for a generalized linear model.[h] Additionally, we assessed the association between serum concentrations of flunarizine (as a continuous variable) and the change in IOP in treated and untreated eyes by use of a generalized linear regression analysis; we also compared these variables by use of a flunarizine concentration adjusted on the basis of body weight (high vs low body weight), in which the median was used as a cutoff point because of the skewed distribution.

The effect of flunarizine on MAP and pupillary diameter was evaluated. A paired *t* test was used to compare values obtained before and after treatment.

Results

IOPs during the baseline period—A significant ($P < 0.001$) intraday fluctuation in IOP was detected, which was highest in the morning and gradually decreased in linear fashion throughout the course of each day (**Figure 1**; **Table 1**). The mean ± SE IOP value for each entire day was significantly ($P = 0.02$) lower for baseline day 2 (14.1 ± 0.54 mm Hg), compared with the value for baseline day 1 (15.0 ± 0.6 mm Hg). Results for baseline day 2 were used in subsequent statistical comparisons because it was likely that the dogs were better adjusted to the manipulations by the second day of baseline data collection.

IOPs during topical treatment with flunarizine—During days 1 and 2 of treatment, IOP did not differ significantly from baseline values for both the treated and untreated eyes (**Table 2**). Starting on treatment day 3 and continuing on days 4 and 5 of flunarizine administration, there was a significant decrease in IOP from baseline values in both treated and untreated eyes. These results were confirmed when results for all 5 treatment days were combined. Changes in IOP differed significantly from baseline values only on days 3, 4, and 5 for both untreated ($P = 0.005$) and treated ($P = 0.009$) eyes and were significantly different from the change in IOP from baseline values on treatment days 1 and 2 for both untreated ($P = 0.01$) and treated ($P = 0.008$) eyes (**Figure 2**). Throughout the entire treatment period, the IOP did not differ significantly ($P = 0.86$) between treated and untreated eyes.

The largest decreases in mean IOP of up to 2.6 mm Hg were during the 1-hour period after the 8 AM drug application. This was especially true for treated eyes in which the mean IOP on treatment days 2 through 5 became consistently smaller from 8 AM to 8:30 AM (Figure 2). After the 2 PM flunarizine treatment, the IOP was not measured for 2 hours, and no obvious short-term decrease in IOP could be detected.

Other clinical variables—Mean ± SE baseline pupillary diameter was 6.5 ± 0.19 mm in both treated and control eyes. Compared with baseline values, there was

Table 1—Mean ± SE IOP measurements for baseline days 1 and 2 combined and baseline day 2 alone for 20 clinically normal dogs that subsequently received unilateral topical administration of flunarizine.

	IOP (mm Hg)	
Time	**Days 1 and 2***	**Day 2†**
8:00 AM	15.8 ± 0.63	15.5 ± 0.83
8:30 AM	15.9 ± 0.52	15.1 ± 0.65
9:00 AM	15.4 ± 0.57	15.1 ± 0.62
9:30 AM	15.0 ± 0.54	14.3 ± 0.40
10:00 AM	14.9 ± 0.57	14.0 ± 0.73
12:00 PM	14.8 ± 0.70	13.9 ± 0.81
2:00 PM	13.9 ± 0.63	13.6 ± 0.72
4:00 PM	13.5 ± 0.70	13.0 ± 0.75
6:00 PM	13.4 ±0.66	13.7 ±0.70
8:00 PM	12.9 ± 0.61	12.6 ± 0.66

,†Within a column, values differed significantly ($P < 0.001$; †$P = 0.004$) among time points as determined by use of a trend test.

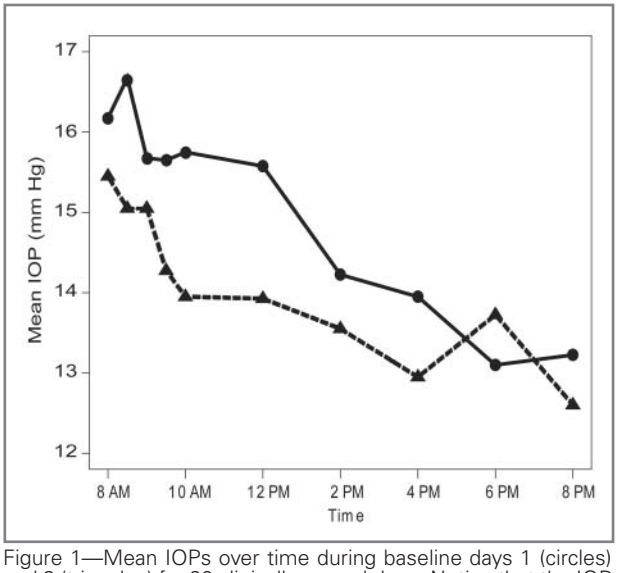

Figure 1—Mean IOPs over time during baseline days 1 (circles) and 2 (triangles) for 20 clinically normal dogs. Notice that the IOP was highest in the morning and gradually decreased throughout the course of each day.

Table 2—Mean ± SE change in IOPs from values for the same times on baseline day 2 during the 5 treatment days for untreated and treated eyes of 20 clinically normal dogs that received unilateral topical administration of flunarizine.

	Untreated eyes*		Treated eyes*	
Treatment day	**Mean ± SE (mm Hg)†**	***P* value‡**	**Mean ± SE (mm Hg)§**	***P* value‡**
1	0.21 ± 0.36	0.57	0.03 ± 0.29	0.93
2	–0.34 ± 0.33	0.30	–0.36 ± 0.32	0.27
3	–0.83 ± 0.33	0.01	–0.76 ± 0.34	0.03
4	–1.10 ± 0.35	0.002	–1.26 ± 0.40	0.002
5	–0.93 ± 0.38	0.01	–0.76 ± 0.40	0.06
1 and 2	–0.07 ± 0.32	0.83	–0.17 ± 0.29	0.57
3, 4, and 5	–0.95 ± 0.34‖	0.005	–0.93 ± 0.35¶	0.009

*Values did not differ significantly ($P > 0.05$) between untreated and treated eyes on any day of treatment. †Values did not differ significantly ($P = 0.09$) among treatment days 1 to 5. ‡Results of statistical tests to determine whether the change of IOP for each treatment day or group of treatment days was equal to 0; values were considered significant at $P \leq 0.05$. §Values differ significantly ($P = 0.02$) among treatment days 1 to 5. ‖Value differs significantly ($P = 0.01$) from the value for days 1 and 2. ¶Value differs significantly ($P = 0.008$) from the value for days 1 and 2.

no significant change in mean pupillary diameter after 5 days of flunarizine administration in treated (6.3 ± 0.18 mm [P = 0.31]) and untreated (6.4 ± 0.16 mm [P = 0.52]) eyes; values for mean pupillary diameter on day 5 of treatment did not differ significantly (P = 0.41) between treated and untreated eyes.

Mean ± SE MAP was 125.1 ± 2.7 mm Hg for all dogs during the baseline period. The MAP did not change significantly (P = 0.71) after the last dose of flunarizine on the final day of treatment (123.4 ± 3.7 mm Hg).

Transient and mild conjunctival hyperemia was evident in the treated eyes of 4 of the 20 dogs after the first treatment time point of the study. Hyperemia resolved within 30 minutes. No other ocular abnormalities were detected in any dogs throughout the duration of the study.

Serum flunarizine concentrations—Flunarizine concentrations were detected in all serum samples obtained 30 minutes after the last treatment (performed at 2 PM on day 5). Serum concentrations ranged from 0.30 to 20.82 µg/L (median, 0.97 µg/L; mean ± SD, 3.89 ± 6.36 µg/L). Change in IOP from baseline values was not significantly associated with the weight-adjusted serum concentrations of flunarizine.

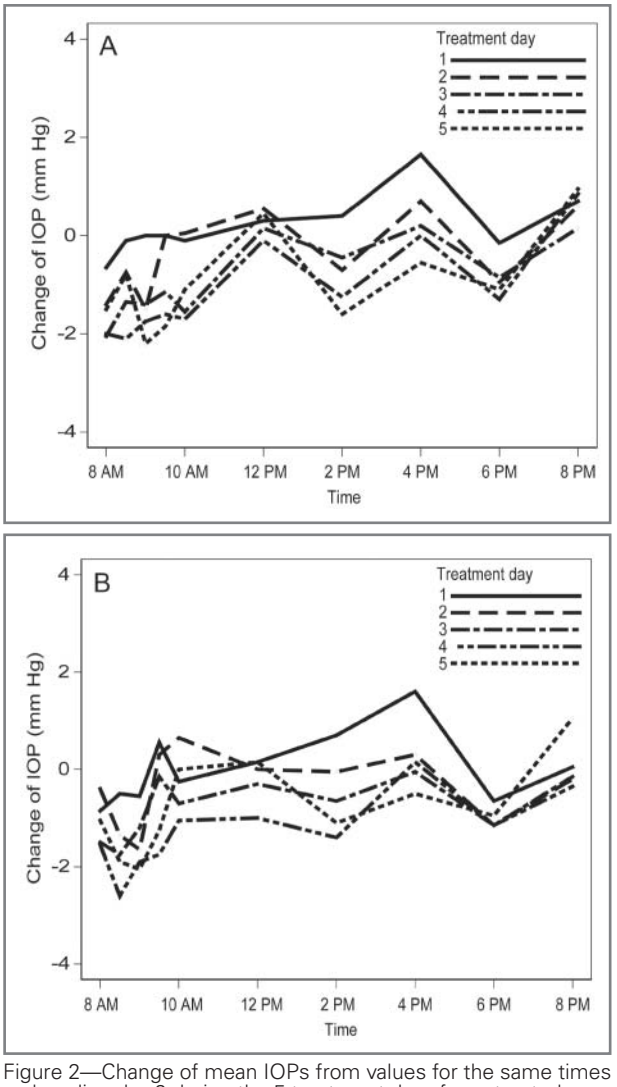

Figure 2—Change of mean IOPs from values for the same times on baseline day 2 during the 5 treatment days for untreated eyes (A) and treated eyes (B) of 20 clinically normal dogs that received unilateral topical administration of flunarizine. Flunarizine (treated eyes) or 50% polyethylene glycol (untreated eyes) was topically applied immediately after IOP measurements were obtained at 8 AM and 2 PM on each of the 5 treatment days. Each line represents results for 1 treatment day.

Discussion

In the study reported here, a significant bilateral decrease in IOP in clinically normal dogs was evident after 2 days of twice-daily unilateral treatment with topically applied 0.5% flunarizine solution. Topical application of flunarizine decreases IOP in rats,[17] rabbits,[13,14,17] and monkeys.[23,b] In our study, the IOP-decreasing effect of topically administered flunarizine became noticeable on treatment day 3 and persisted for the remainder of the study. The treatment effect was most dramatic during the 1-hour time interval immediately after the 8 AM drug application, especially in treated eyes, with mean IOP decreases of up to 2.6 mm Hg (Figure 2). The decrease in IOP was less obvious after the 2 PM flunarizine application. We suspect that a larger decrease in IOP during the afternoon was missed because IOP measurements were not performed every 30 minutes as they were in the morning.

Studies[13,14] in albino rabbits also revealed a maximal decrease in IOP of 2 to 3 mm Hg by 1 hour after a single topical application of flunarizine. In those studies, IOP returned to baseline values within 4 hours after treatment. In contrast to the results in rabbits, there was no significant decrease in IOP after the first application of flunarizine in the dogs of our study.

Our results are more consistent with results obtained for clinically normal monkeys[b] in which a decrease in IOP was not detected with twice-daily topical application of 0.5% flunarizine until the third day of treatment. In glaucomatous monkeys,[a] a dose-dependent decrease in IOP was detected after topical application of flunarizine (0.5% to 2%). The ocular hypotensive effect was enhanced with twice-daily administration of 0.5% flunarizine for 5 days.[a] In those glaucomatous monkeys, the maximum mean reduction in IOP increased from 2.5 mm Hg on day 1 to 6 mm Hg on day 5 of treatment. In glaucomatous eyes of rabbits, a mean maximum IOP decrease of 12 mm Hg was detected with a 1-time topical application of flunarizine.[14] It is likely that flunarizine could also lead to a much larger decrease in IOP in glaucomatous eyes of dogs, compared with the effect in the normotensive eyes tested in the study reported here.

A 2-day baseline period allowed us to compare the effect of flunarizine on IOP against established typical diurnal IOPs. There was a significant difference in IOP between baseline days 1 and 2. This difference was probably attributable to the process of establishing a uniform operating protocol and organization and allowing for the dogs' adjustments to manipulation. Therefore, values for baseline day 2 were chosen for comparison with treatment values. A rebound tonometer was used to measure the IOPs in our study. The accuracy and validity of this instrument have been reported[28,29] for determining IOP in dogs.

Intraday fluctuation of IOP with highest values in the morning that decrease throughout the day is consistent with diurnal IOP changes for dogs that have been

reported elsewhere.[30-32] When averaged for baseline days 1 and 2, the highest mean IOP was measured at 9 AM (15.8 mm Hg) and it decreased by 2.9 mm Hg to the lowest value at 8 PM (12.9 mm Hg). This decrease in IOP was comparable to the 2.5 mm Hg decrease reported[30] in glaucomatous eyes of Beagles.

Throughout the study, there were no significant differences in IOP between treated and untreated eyes, which suggested a systemic effect of flunarizine on the untreated eye in addition to the local effect on the treated eye. A decrease in the IOP of the untreated contralateral eye has also been reported in rabbits,[18] monkeys,[23,a] and human patients[33] treated topically with calcium-channel blockers, including flunarizine. Similar to findings in a study[13] in rabbits, a systemic effect was supported in our dogs by the detection of flunarizine in all serum samples obtained 30 minutes after the last drug application. Overall, serum concentrations were variable among the dogs in the study, but there was no significant correlation between these drug concentrations and the IOP decrease in a particular dog. However, without knowledge about flunarizine pharmacokinetics in dogs after topical application, the lack of such a correlation between serum concentrations at an arbitrary time point and the extent of IOP reduction does not rule out a systemic effect.

In 1 study,[13] investigators measured flunarizine concentrations in ocular tissue compartments and plasma concentrations in albino rabbits following topical application and found that plasma flunarizine concentrations were highest 15 minutes after instillation and were still detectable at 30 minutes but were not detectable beyond 60 minutes. No correlations were made between serum concentrations and IOP-decreasing effects in that study.[13] After a single topical application of 0.05% flunarizine, investigators were able to detect the drug at 15 minutes (first time point) in the cornea and at 30 minutes (second time point) in the aqueous humor, uveal tract, and retina.[13]

Although we believe that the bilateral decrease in mean IOP was most likely caused by the unilateral topical application of flunarizine, other possibilities must be considered. The decrease in IOP from baseline day 1 to baseline day 2 could have continued during the treatment period because the dogs were continuing to become more adjusted to the experimental procedures. Continued tonometry during a washout period of several days would have helped to rule out such a continued learning effect, assuming the IOPs returned to baseline values after discontinuation of treatment. Although we cannot rule out such a learning effect on the basis of the available data, it seems unlikely that it could explain the more acute decrease in IOP after the 8 AM flunarizine applications.

Another possible explanation for the bilateral IOP decrease could have been an ocular hypotensive effect of the polyethylene glycol vehicle. We believe that this was unlikely because there are no reports for such an effect in the literature. Polyethylene glycol has been used by others to compound drugs for topical application and for evaluation of ocular hypotensive effects.[34] Unfortunately, we did not have a control group of dogs treated with the polyethylene glycol vehicle alone to evaluate its effect on IOP.

Both polyethylene glycol and flunarizine could have decreased the IOP by causing mild subclinical anterior uveitis. This would be similar to results for prostaglandin analogues or the parasympathomimetic pilocarpine with ocular hypotensive effects associated with clinical signs of anterior uveitis in dogs.[35,36] Despite the lack of miosis and aqueous flare, we could not rule out anterior uveitis in the dogs of our study.

Flunarizine is a mixed L- and T-type calcium-channel blocker[37] with additional antagonistic effects on sodium channels[38] and mixed agonist-antagonist action on opioid receptors.[14,39] Several mechanisms have been discussed for the IOP-decreasing effect of calcium-channel blockers, including a decrease in production of aqueous humor by inhibition of calcium-dependent cation transport in the nonpigmented ciliary epithelium[40] and an increase in outflow facilitated by relaxation of trabecular meshwork cells.[41-43,b]

Although the pH of the drug solution was low (pH, 1.8), application of the drug resulted in only 4 of 20 dogs having signs of mild and transient conjunctival hyperemia in the treated eyes that lasted for < 30 minutes and did not recur with subsequent treatments. No other clinical signs of ocular irritation were detected during the study in any of the dogs, and no evidence of inflammation in the anterior segment was evident during slit-lamp biomicroscopy. Similar concentrations of flunarizine dissolved in polyethylene glycol have been evaluated, but investigators did not report the final pH of the drug.[17,a,b] In 1 study,[a] investigators found that monkeys subjected to topical administration of a 2% preparation of flunarizine solution had signs of mild blepharoconjunctivitis and infrequent corneal edema. Because these same authors[a,b] detected a significant reduction in IOP with minimal adverse effects in monkeys with the use of a 0.5% solution of flunarizine, we chose the same drug concentration. We have not noticed any adverse systemic effects in the clinically normal dogs used in our study. Even though unlikely, it is not clear whether the effect of the low-pH solution was partially responsible for small changes in IOP. In a study[36] on the effect of topically applied pilocarpine on the IOP in dogs, investigators reported that solutions with a low pH led to a brief increase in IOP (rather than a decrease in IOP) during the first hour after drug instillation. One goal of future studies should be to improve the formulation of flunarizine for topical application to make it less irritating.

Analysis of results of the study reported here suggested a bilateral ocular hypotensive effect of unilateral topical administration of 0.5% flunarizine after 2 days of twice-daily application in clinically normal dogs. This calcium-channel blocker should also be evaluated for its IOP-decreasing and neuroprotective effects in dogs with glaucoma.[10,17,25]

a. Wang RF, Gagliuso DJ, Mittag TW, et al. Effect of flunarizine, a calcium channel blocker, on intraocular pressure (IOP) in glaucomatous monkey eyes (abstr), in *Proceedings*. Assoc Res Vis Ophthalmol Annu Meet 2005;3778.
b. Wang RF, Gagliuso DJ, Podos SM. Effect of flunarizine on aqueous humor dynamics in monkeys (abstr), in *Proceedings*. Assoc Res Vis Ophthalmol Annu Meet 2006;396.
c. TonoVet, type TA 01, Tiolat, Helsinki, Finland.
d. Sigma-Aldrich, St Louis, Mo.

e. Cardell indirect blood pressure system, Sharn Veterinary Inc, Tampa, Fla.
f. Costar Spin-X, Corning Inc, Corning, NY.
g. LCQ Deca XP Plus with a surveyor MS pump and autosampler plus, Thermo Fisher Scientific Inc, Waltham, Mass.
h. PROC GENMOD, SAS, version 9.1, SAS Institute Inc, Cary, NC.

References

1. Quigley HA, Broman AT. The number of people with glaucoma worldwide in 2010 and 2020. *Br J Ophthalmol* 2006;90:253–254.
2. Gelatt KN, MacKay EO. Prevalence of the breed-related glaucomas in pure-bred dogs in North America. *Vet Ophthalmol* 2004;7:97–111.
3. Fechtner RD, Weinreb RN. Mechanisms of optic nerve damage in primary open angle glaucoma. *Surv Ophthalmol* 1994;39:23–42.
4. Flammer J, Orgül S. Optic nerve blood-flow abnormalities in glaucoma. *Prog Retin Eye Res* 1998;17:267–289.
5. Gelatt KN, Miyabayashi T, Gelatt-Nicholson KJ, et al. Progressive changes in ophthalmic blood velocities in Beagles with primary open angle glaucoma. *Vet Ophthalmol* 2003;6:77–84.
6. Brooks DE, Garcia GA, Dreyer EB, et al. Vitreous body glutamate concentration in dogs with glaucoma. *Am J Vet Res* 1997;58:864–867.
7. Schwartz M. Neurodegeneration and neuroprotection in glaucoma: development of a therapeutic neuroprotective vaccine—the Friedenwald lecture. *Invest Ophthalmol Vis Sci* 2003;44:1407–1411.
8. Weinreb RN, Khaw PT. Primary open-angle glaucoma. *Lancet* 2004;363:1711–1720.
9. Netland PA, Chaturvedi N, Dreyer EB. Calcium channel blockers in the management of low-tension and open-angle glaucoma. *Am J Ophthalmol* 1993;115:608–613.
10. Takahashi K, Lam TT, Edward DP, et al. Protective effects of flunarizine on ischemic injury in the rat retina. *Arch Ophthalmol* 1992;110:862–870.
11. Saito S, Ohashi M, Naito A, et al. Neuroprotective effect of the novel Na+/Ca2+ channel blocker NS-7 on rat retinal ganglion cells. *Jpn J Ophthalmol* 2005;49:371–376.
12. Ishii K, Fukaya Y, Araie M, et al. Topical administration of iganidipine, a new water-soluble Ca2+ antagonist, increases ipsilateral optic nerve head circulation in rabbits and cynomolgus monkeys. *Curr Eye Res* 2004;29:67–73.
13. Maltese A, Bucolo C. Pharmacokinetic profile of topical flunarizine in rabbit eye and plasma. *J Ocul Pharmacol Ther* 2003;19:171–179.
14. Campana G, Bucolo C, Murari G, et al. Ocular hypotensive action of topical flunarizine in the rabbit: role of sigma 1 recognition sites. *J Pharmacol Exp Ther* 2002;303:1086–1094.
15. Beatty JF, Krupin T, Nichols PF, et al. Elevation of intraocular pressure by calcium channel blockers. *Arch Ophthalmol* 1984;102:1072–1076.
16. Melena J, Santafe J, Segarra J. The effect of topical diltiazem on the intraocular pressure in betamethasone-induced ocular hypertensive rabbits. *J Pharmacol Exp Ther* 1998;284:278–282.
17. Osborne NN, Wood JP, Cupido A, et al. Topical flunarizine reduces IOP and protects the retina against ischemia-excitotoxicity. *Invest Ophthalmol Vis Sci* 2002;43:1456–1464.
18. Segarra J, Santafe J, Garrido M, et al. The topical application of verapamil and nifedipine lowers intraocular pressure in conscious rabbits. *Gen Pharmacol* 1993;24:1163–1171.
19. Tamaki Y, Araie M, Fukaya Y, et al. Effects of lomerizine, a calcium channel antagonist, on retinal and optic nerve head circulation in rabbits and humans. *Invest Ophthalmol Vis Sci* 2003;44:4864–4871.
20. Tamaki Y, Araie M, Tomita K, et al. Time change of nicardipine effect on choroidal circulation in rabbit eyes. *Curr Eye Res* 1996;15:543–548.
21. Harino S, Riva CE, Petrig BL. Intravenous nicardipine in cats increases optic nerve head but not retinal blood flow. *Invest Ophthalmol Vis Sci* 1992;33:2885–2890.
22. Källberg ME, Brooks DE, Komaromy AM, et al. The effect of an L-type calcium channel blocker on the hemodynamics of orbital arteries in dogs. *Vet Ophthalmol* 2003;6:141–146.
23. Siegner SW, Netland PA, Schroeder A, et al. Effect of calcium channel blockers alone and in combination with antiglaucoma medications on intraocular pressure in the primate eye. *J Glaucoma* 2000;9:334–339.
24. Netland PA, Grosskreutz CL, Feke GT, et al. Color Doppler ultrasound analysis of ocular circulation after topical calcium channel blocker. *Am J Ophthalmol* 1995;119:694–700.
25. Osborne NN, Ugarte M, Chao M, et al. Neuroprotection in relation to retinal ischemia and relevance to glaucoma. *Surv Ophthalmol* 1999;43(suppl 1):S102–S128.
26. Cellini M, Possati GL, Caramazza N, et al. The use of flunarizine in the management of low-tension glaucoma: a color Doppler study. *Acta Ophthalmol Scand Suppl* 1997;224:57–58.
27. Munger RJ. Veterinary ophthalmology in laboratory animal studies. *Vet Ophthalmol* 2002;5:167–175.
28. Knollinger AM, La Croix NC, Barrett PM, et al. Evaluation of a rebound tonometer for measuring intraocular pressure in dogs and horses. *J Am Vet Med Assoc* 2005;227:244–248.
29. Leiva M, Naranjo C, Pena MT. Comparison of the rebound tonometer (ICare) to the applanation tonometer (Tonopen XL) in normotensive dogs. *Vet Ophthalmol* 2006;9:17–21.
30. Gelatt KN, Gum GG, Barrie KP, et al. Diurnal variations in intraocular pressure in normotensive and glaucomatous Beagles. *J Glaucoma* 1981;3:121–124.
31. Wilkie DA, Latimer CA. Effects of topical administration of timolol maleate on intraocular pressure and pupil size in dogs. *Am J Vet Res* 1991;52:432–435.
32. Skorobohach BJ, Ward DA, Hendrix DV. Effects of oral administration of methazolamide on intraocular pressure and aqueous humor flow rate in clinically normal dogs. *Am J Vet Res* 2003;64:183–187.
33. Abelson MB, Gilbert CM, Smith LM. Sustained reduction of intraocular pressure in humans with the calcium channel blocker verapamil. *Am J Ophthalmol* 1988;105:155–159.
34. Colasanti BK, Brown RE, Craig CR. Ocular hypotension, ocular toxicity, and neurotoxicity in response to marihuana extract and cannabidiol. *Gen Pharmacol* 1984;15:479–484.
35. Gelatt KN, MacKay EO. Effect of different dose schedules of latanoprost on intraocular pressure and pupil size in the glaucomatous Beagle. *Vet Ophthalmol* 2001;4:283–288.
36. Gelatt KN, Mackay EO, Gelatt JK, et al. Effects on intraocular pressure and pupil size in glaucomatous beagles after topical pilocarpine instilled with standard (pH 5) and buffer-tip (pH 7) droptainers. *J Ocul Pharmacol Ther* 1997;13:95–104.
37. Holmes B, Brogden RN, Heel RC, et al. Flunarizine. A review of its pharmacodynamic and pharmacokinetic properties and therapeutic use. *Drugs* 1984;27:6–44.
38. Pauwels PJ, Leysen JE, Janssen PA. Ca^{2+} and Na^{+} channels involved in neuronal cell death. Protection by flunarizine. *Life Sci* 1991;48:1881–1893.
39. Weizman R, Getslev V, Pankova IA, et al. Pharmacological interaction of the calcium channel blockers verapamil and flunarizine with the opioid system. *Brain Res* 1999;818:187–195.
40. Mito T, Delamere NA, Coca-Prados M. Calcium-dependent regulation of cation transport in cultured human nonpigmented ciliary epithelial cells. *Am J Physiol* 1993;264: C519–C526.
41. Lepple-Wienhues A, Rauch R, Clark AF, et al. Electrophysiological properties of cultured human trabecular meshwork cells. *Exp Eye Res* 1994;59:305–311.
42. Lepple-Wienhues A, Stahl F, Wiederholt M. Differential smooth muscle-like contractile properties of trabecular meshwork and ciliary muscle. *Exp Eye Res* 1991;53:33–38.
43. Erickson KA, Schroeder A, Netland PA. Verapamil increases outflow facility in the human eye. *Exp Eye Res* 1995;61:565–567.