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Effect of different dose schedules of travoprost on intraocular pressure and pupil size in the glaucomatous Beagle

Kirk N. Gelatt and Edward O. MacKay

Department of Small Animal Clinical Sciences and Gwathmey-Adams Laboratory for Vision Science, College of Veterinary Medicine, University of Florida, Gainesville, FL 32610–0126, USA

Address communications to:

K. N. Gelatt

Tel.: (352) 392–4700
Fax: (352) 392–6125
e-mail:
Gelattk@mail.vetmed.ufl.edu

Abstract

Objective To evaluate changes in intraocular pressure and pupil size in glaucomatous dogs after instillation of 0.004% travoprost once in the morning, or once in the evening, or twice daily in 5-day multiple dose studies.

Materials and methods Applanation tonometry (IOP) and pupil size (PS) measurements were obtained at 8 a.m., 10 a.m., 12 noon, 2 p.m. and 4 p.m. in eight glaucoma dogs. Methylcellulose (0.5% as placebo) was instilled in the control eye, and 0.004% travoprost was instilled in the opposite drug eye. Methylcellulose (0.5%) and 0.004% travoprost were instilled on the 2nd through to the 5th day with instillations in the morning (8.30 a.m.), or evening (8 p.m.), or twice daily (8.30 a.m. and 8 p.m.).

Results The mean ± SEM diurnal changes from baseline IOP in the control and placebo eyes in all three studies ranged from 1.2 ± 0.3 mmHg to 3.2 ± 0.9 mmHg. The mean ± SEM diurnal changes from the baseline IOP after 0.004% travoprost at 8 a.m. once daily for the next 4 days were 19.0 ± 2.7 mmHg, 24.7 ± 2.7 mmHg, 24.9 ± 3.1 mmHg, and 24.7 ± 3.1 mmHg, respectively, and were significantly different from the control eye. After travoprost was instilled at 8 p.m., the mean ± SEM baseline changes from the baseline IOP in the drug eyes were 23.5 ± 2.2 mmHg, 24.2 ± 2.2 mmHg, 24.5 ± 2.3 mmHg, and 24.2 ± 2.3 mmHg, respectively. When 0.004% travoprost was instilled twice daily, the mean ± SEM baseline IOP changes were 27.7 ± 2.1 mmHg, 28.1 ± 2.1 mmHg, 28.4 ± 2.2 mmHg, and 28.5 ± 2.2 mmHg, respectively, and were significantly different from the control eyes. Miosis of varying duration was frequent during the three studies.

Conclusion Travoprost instilled once daily (a.m. or p.m.) as well as twice daily produces significant decreases in IOP and PS in the glaucomatous Beagle.

Key Words: dog, glaucoma, prostaglandin, travoprost

INTRODUCTION

With the introduction of timolol in 1978, medical therapy of the glaucomas in humans changed from cholinergic miotics and epinephrine compounds to mainly beta antagonists.[1] However, because of their action and systemic absorption these beta adrenergic blockers can be contraindicated in patients with certain cardiac and pulmonary diseases.[2] Starting in the 1990s, the introduction of topical prostaglandins (PGs), i.e. latanoprost (Xalatan, Pharmacia, Kalmazoo, MI, USA), unoprostone isopropyl (Rescula, Ciba Vision, Duluth, GA, USA), bimatoprost (Lumigan, Allergan, Irvine, CA, USA) and travoprost (Travatan, Alcon Laboratories, Ft Worth, TX, USA), provided a new class of topical ocular hypotensive drugs for clinical management of the glaucomas in humans.[3–8] These agents offer the advantage of single drop administration daily, their ocular hypotensive effects are additive to other glaucoma drugs, and they avoid the cardiopulmonary effects of the beta blockers.[1] Their primary adverse effects with chronic use in humans, nonhuman primates and rabbits are increased iridal pigmentation, hypertrichosis and pigmentation of the eyelashes.[9–11]

PGs have been shown to reduce IOP in a number of animal species including dogs, cats, nonhuman primates and rabbits by mainly increasing uveoscleral aqueous humor outflow; however, the amount of IOP reduction can vary by the individual PG as well as the species.[12–19] In humans reductions in IOP occur with PGE and PGF analogs[3,5] and similar

IOP declines occur in the normal and glaucomatous dog after topical PGA_2 isopropyl ester and $PGF_{2\mu}$ isopropyl ester, but not after PGA_2.[17,18]

The IOP of normal and glaucomatous dogs has significant reductions after several PGs. In normotensive and glaucomatous Beagles, two drugs similar to latanoprost ($PGF_{2\alpha}$-17-diphenyl and $PGF_{2\alpha}$-isopropyl ester) lowered IOP, with the greatest decline occurring in the glaucomatous dogs.[13,17] In a study in normal dogs, 0.005% latanoprost decreased IOP (mean: 3 mmHg or 25%),[18,19] and in the glaucomatous Beagle reduced IOP from 40 to 60%.[20] Unoprostone isopropyl (0.12%) in normal dogs lowers IOP, and the reduction is about 5 mmHg (25%).[21] Brimatoprost in the glaucomatous Beagle induced marked reductions in IOP (up to 78%) that often persisted longer that 24 h after a single instillation.[22]

Travoprost, introduced in 2001, is a isopropyl ester of a single enantiomer of the selective FP prostaglandin receptor agonist, fluprostenol.[23–25] Clinical reports of travoprost in humans are limited, but the drug's efficacy in lowering IOP appears similar to latanoprost.[26–29] In this study three different dose schedules with 0.004% travoprost were evaluated in glaucomatous Beagles. The drug was instilled once daily in the morning, once daily in the evening, and twice daily (morning and evening), and the effects on intraocular pressure (IOP) and pupil size (PS) were measured.

MATERIALS AND METHODS

Eight glaucomatous Beagles were used (four males: 44 (2 dogs), 92 and 135 months old; four females: 45, and 92 (3 dogs) months old). The three different dosing studies were evaluated in random order and included: placebo (0.5% methylcellulose), and 0.004% travoprost (Travatan, Alcon, Ft Worth, TX, USA). All dogs were examined by slit-lamp biomicroscopy, gonioscopy, and ophthalmoscopy, exhibited primary open angle glaucoma in the moderate stage (narrow iridocorneal angle and ciliary cleft opening), and demonstrated clinical vision.

Recorded measurements included IOP by applanation tonometry (Tonopen-XL, Mentor O and O, Norwell, MA, USA), and PS measurements by Jameson calipers. The study times for the three dose studies were one day of control and no drug, and then four days of placebo and drug instillations, followed by 14 days to allow for drug washout between each study. The drug eye for each dog was selected at random, and all measurements were at 8 a.m., 10 a.m., 12 noon, 2 p.m. and 4 p.m. For the first once-daily dose study, drug and placebo were instilled at 8.30 a.m. on the second day; for the second once-daily dose study, drug and placebo were instilled at 8 p.m. at the end of the first day; and for the last twice-daily dose study, drug and placebo were instilled at 8.30 a.m. and 8 p.m. on the second day.

Drug comparisons were performed using SAS programs utilizing Tukey's HSD and ANOVA tests for repeated measurements.[30] Within each test week, the average measurements for IOP, and PS for each day were compared with subsequent measurements to detect significant changes ($P < 0.05$) using the Tukey and Duncan tests and ANOVA for repeated measurements. Between the control and the drug (0.5% methylcellulose or 0.004% travoprost) eyes, the changes in IOP and PS were compared for each measurement between the groups. Statistical significance was $P < 0.05$.

RESULTS

Single morning dose study

The mean ± SEM changes in IOP and PS for the control and drug eyes for the first day are summarized in Fig. 1a and b. The baseline 8 a.m. IOP for the control eye was 42.2 ± 2.8 mmHg with a diurnal decline of 2.0 ± 0.5 mmHg through the day. In the drug eyes similar IOP changes occurred (2.6 ± 0.5 mmHg), starting with a baseline (8 a.m.) IOP of 41.7 ± 2.8 mmHg. There were no significant differences between the control and placebo eyes ($P = 0.882$) throughout the 7.5 h of observation. Mean ± SEM PS changes (control:

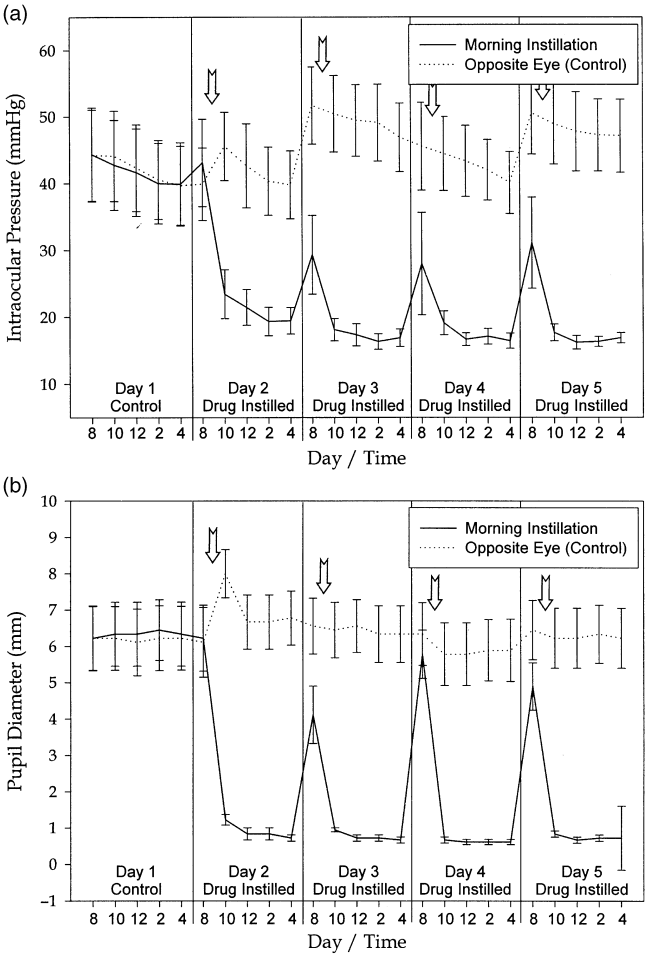

Figure 1. (a) Mean ± SEM intraocular pressure changes after 0.004% travoprost instillations (⇓) at 8.30 a.m. from day 2 to day 5 in glaucomatous Beagles. (b) Mean ± SD changes in pupil size after 0.004% travoprost instillations (⇓) at 8.30 a.m. from day 2 to day 5 in glaucomatous Beagles.

6.2 ± 0.4 mm and placebo: 6.3 ± 0.4 mm) were also not significant ($P = 0.212$) throughout the day.

For the following 4 days, IOP and PS changes (mean ± SEM) from baseline levels after 0.004% travoprost instillation in one eye of each dog each morning are summarized. Comparisons of the nondrug and 0.004% travoprost eyes indicated significant IOP differences with mean ± SEM diurnal changes for the drug eyes of 19.0 ± 2.7 mmHg, 24.7 ± 2.7 mmHg, 24.9 ± 3.1 mmHg, and 24.7 ± 3.1 mmHg (day 2 to day 5, respectively) starting at the first 10 a.m. measurement of day 2 and continuing for all measurements to the end of day 5. Comparisons of the IOP changes between the control eyes of the first day, and these same eyes for the following 4 days also indicated significant changes ($P = 0.0001$).

The miosis that developed in the 0.004% travoprost eyes was significantly different ($P = 0.0001$) from the fellow control eye occurring at the second hour of measurements of the second day, and lasted throughout the remaining 4 days.

Single evening dose study

The IOP and PS changes (mean ± SEM) from baseline levels after 0.004% travoprost instillations at 8 p.m. are summarized in Fig. 2a and b. For the first day, the mean ± SEM diurnal IOP changes in the placebo and control eyes were 1.5 ± 1.9 mmHg and 1.2 ± 0.3 mmHg, respectively. Comparisons of the nondrug and drug eyes indicated that the first-day IOP and PS changes were not significantly different ($P = 0.871$ and $P = 0.834$, respectively) at all five measurement times.

For the following 4 days of 8 p.m. 0.004% travoprost instillations, significant changes in IOP and PS occurred for all measurement times between the control and drug eyes starting at the first 8 a.m. measurement after drug instillation on day 2. The baseline IOP changes of the drug eye were 23.5 ± 2.2 mmHg, 24.2 ± 2.2 mmHg, 24.5 ± 2.3 mmHg, and 24.2 ± 2.3 mmHg, respectively. Daily fluctuations in IOP were limited or absent. The PS changes were about the same duration as the IOP reductions.

Twice daily (morning and evening) dose study

The IOP and PS changes from baseline levels after 0.004% travoprost instillations (8.30 a.m. and 8 p.m.) are summarized in Fig. 3a and b. During the first day of control, the mean ± SEM diurnal IOP changes in the placebo and control eyes were 3.8 ± 1.6 mmHg and 3.2 ± 0.9 mmHg, respectively. Comparison of the control to the drug eyes indicated no significant difference ($P = 0.833$). The pupil size also remained reasonably constant, and no significant changes occurred throughout the first day ($P = 0.968$).

However, with 0.004% travoprost instillations at 8 p.m. on day 1, and the next 4 days at 8.30 a.m. and 8 p.m., the IOP and PS changes were significantly different, starting with first measurements (8 a.m.) between the drug and placebo eyes on day 2 and continuing thereafter. The IOP reductions were more uniform, and daily fluctuations or spikes were of limited magnitude or absent, and for the drug eye were

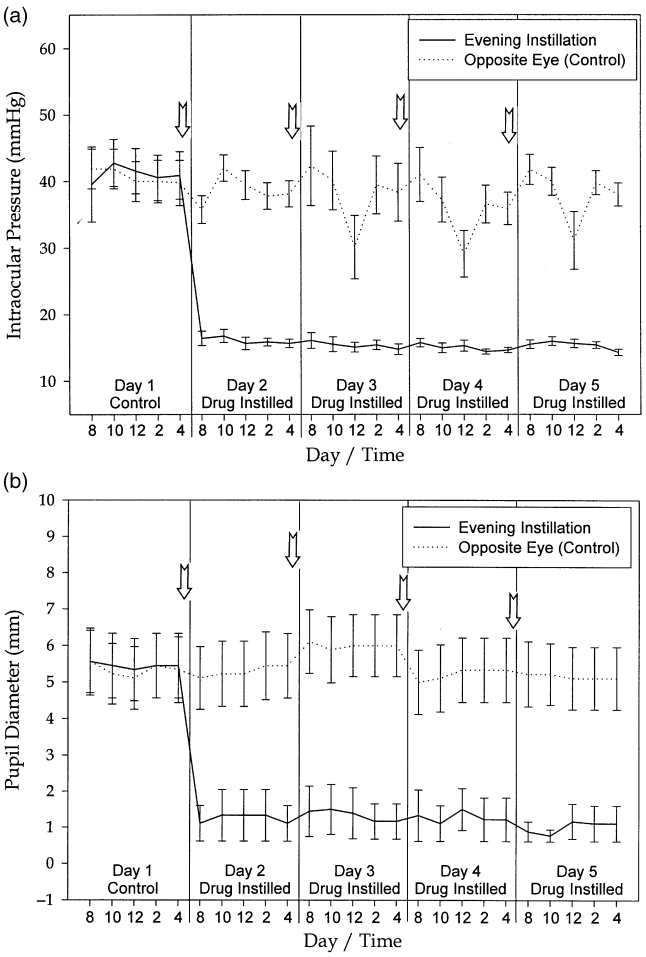

Figure 2. (a) Mean ± SEM intraocular pressure changes after 0.004% travoprost instillations (⇓) at 8 p.m. from day 1 to day 5 in glaucomatous Beagles. (b) Mean ± SD pupil size changes after 0.004% travoprost instillations (⇓) at 8 p.m. from day 1 to day 5 in glaucomatous Beagles.

27.7 ± 2.1 mmHg, 28.1 ± 2.1 mmHg, 28.4 ± 2.2 mmHg, and 28.5 ± 2.2 mmHg for days 2 to 5, respectively.

Significant changes also occurred in PS of the drug eyes resulting in miosis after 8 a.m. of the second day and continuing throughout the remaining measurements of the 4 days ($P = 0.0001$). Comparison of the IOP changes of the three different dosing schedules indicated the twice daily schedule produced a significantly lower IOP and the least IOP fluctuations for all measurements ($P = 0.015$). Comparison of the once-daily morning and evening instillations did not reveal significant differences ($P = 0.900$).

DISCUSSION

Past prostaglandin studies in both normal and glaucomatous dogs indicate the ocular hypertensive dogs appear more sensitive and demonstrate greater declines in IOP than normotensive dogs.[13,17,21] Latanoprost using normal dogs produced a mean decline in IOP of 3 mmHg or about 25%,[19] but with glaucomatous dogs the average decline in IOP was

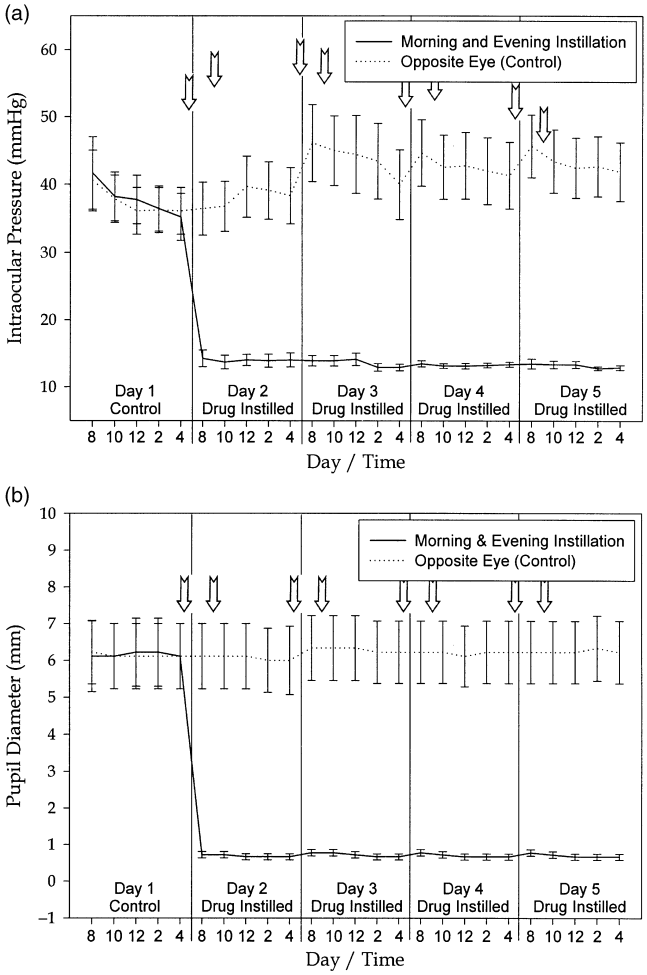

Figure 3. (a) Mean ± SEM intraocular pressure changes after 0.004% travoprost instillations (⇓) at 8 p.m. on day one, and 8.30 a.m. and 8 p.m. from day 2 to day 5 in glaucomatous Beagles. (b) Mean ± SD changes in pupil size after 0.004% travoprost instillations (⇓) at 8 p.m. on day 1, and 8.30 a.m. and 8 p.m. from day 2 to day 5 in glaucomatous Beagles.

about 50% for 1 day of drug administration, and 50% to 78% after 4 days of twice daily latanoprost and brimatoprost administrations.[20,22]

In this study 0.004% travoprost instilled in the morning, or evening, or twice daily produced significant decreases in IOP, and variable miosis. With the once daily 0.004% travoprost in the morning, the daily reduction in IOP of the drug eyes changed from 6.2% (day 1; 8 a.m.; no drug) to 59.2% (the last of 4 days of drug). When 0.004% travoprost was instilled in the evening of day 1, the decline in IOP for the following first day was 3.6%, and after 4 days of drug instillations the IOP had declined 58.8% from the baseline of day 1. When 0.004% travoprost was instilled in both the morning and evening of day 2, the IOP reduction for the first day of drug administration was 10.0%, and after 4 days of drug instillation, the reduction in IOP was 75.2% compared to the IOP of day 1, 8 a.m.

The different dose schedules of travoprost also affected the diurnal or circadian IOP cycle in the glaucomatous dogs, and the daily IOP fluctuations or spikes. In both normal and glaucomatous dogs the highest IOP occurs in the morning.[31] Daily spikes in IOP occurred with the single morning instillations, but were not detected when 0.004% travoprost was instilled in the evening or twice daily. The daily spikes in IOP may potentially cause more harm to the retinal ganglia cells and optic nerve head,[32] and 'smoothing out' these daily IOP fluctuations may also be beneficial in the dog. The canine isolated sphincter muscle appears sensitive to PGFα, which may act directly rather than through the release of adrenergic or cholinergic neurotransmitters.[33,34] The large decline in IOP as well as occurrence of miosis with the marked declines in IOP following twice-daily instillations of 0.004% travoprost in the glaucomatous dogs suggests trabecular outflow as well as uveoscleral outflow may be increased.

As these drugs are analogs of PGs, they produce ocular irritation, conjunctival hyperemia, breakdown in the blood–aqueous barrier, aqueous flare, and miosis in the dog.[35] The evening instillations of 0.004% travoprost minimize these effects the following morning.

Based on several drug studies of the glaucomatous Beagles, the greatest decline in IOP occurred after topical direct and indirect cholinergic miotics, such as pilocarpine, demecarium, and echothiophate.[36,37] Compared to the topical beta-adrenoreceptor antagonists and topical carbonic anhydrase inhibitors,[38,39] latanoprost, brimatoprost and travoprost offer a greater and most prolonged reduction in IOP for the dog.

In humans, nonselective beta-blockers lower IOP by 4–6 mmHg (12–25%); the once-daily prostaglandins (latanoprost, bimatoprost and travoprost) lower IOP 6–8 mmHg (up to 50%); and the topical carbonic anhydrase inhibitors reduce IOP about 20–30%.[1] In the glaucomatous Beagle these prostaglandins lower IOP 40–76%, and the reduction in mmHg ranges from 24 to 40 mmHg.[17,20,22] Of the currently available drugs to treat the canine glaucomas, the topical prostaglandins can markedly lower IOP.

In summary, 0.004% travoprost significantly lowers IOP in the glaucomatous Beagle when instilled in the evening, morning, or twice daily. Miosis is usually present, but more limited when travoprost was instilled only in the evening. The evening and twice-daily instillations of travoprost result in the least IOP fluctuations. The reduction in actual IOP ranged from about 24.2 mmHg (58.8%) to 28.5 mmHg (76%).

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REFERENCES

1. Soltau JB, Zimmerman TJ. Changing paradigms in the medical treatment of glaucoma. *Survey Ophthalmology* 2002; **47**: S2–S5.
2. Lama PJ. Systemic adverse effects of beta-adrenergic blockers: an evidence-based assessment. *American Journal of Ophthalmology* 2002; **134**: 749–760.

3. Higginbotham EJ. (ed.) Will latanoprost be the 'wonder' drug of the '90s for the treatment of glaucoma? *Archives of Ophthalmology* 1996; **114**: 998–999.
4. Wyse TB, Talluto DM, Krupin T *et al.* Topical prostaglandins for glaucoma therapy. *Journal of Glaucoma* 1997; **6**: 180–187.
5. Bito LZ. Prostaglandins: a new approach to glaucoma management with a new intriguing side effect. *Survey of Ophthalmology* 1997; **41** (Suppl. 2): S1–S14.
6. Alm A, Stjernschantz J. Effects on intraocular pressure and side effects of 0.005% latanoprost applied once daily, evening or morning. *Ophthalmology* 1995; **102**: 1743–1752.
7. Susanna R, Giampani J, Borges AS *et al.* A double-masked randomized clinical trail comparing latanoprost with unoprostone in patients with open-angle glaucoma or ocular hypertension. *Ophthalmology* 2001; **108**: 259–263.
8. Gandolfi S, Simmons ST, Sturm R *et al.* Three-month comparison of bimatoprost and latanoprost in patients with glaucoma and ocular hypertension. *Advances in Therapy* 2001; **18**: 110–121.
9. Zhan GL, Toris CB, Camras CB *et al.* Prostaglandin-induced iris color darkening. *Archives of Ophthalmology* 1998; **116**: 1065–1068.
10. Selen G, Stjernschantz J, Resul B. Prostaglandin-induced iridal pigmentation in primates. *Survey of Ophthalmology* 1997; **41** (Suppl.): S125–S128.
11. Zhan GL, Toris CB, Camras CB *et al.* Prostaglandin-induced iris color darkening. *Archives of Ophthalmology* 1998; **116**: 1065–1068.
12. Lee P, Podos SM, Severin C. Effect of prostaglandin $F_{2\alpha}$ on aqueous humor dynamics of rabbit, cat, and monkey. *Investigative Ophthalmology Visual Science* 1984; **25**: 1087–1093.
13. Bito LZ, Camras CB, Gum GG, Resul B. The ocular hypotensive effects and side effects of prostaglandins on the eyes of experimental animals. In: *The Ocular Effects of Prostaglandins and Other Eicosanoids.* (eds Bito LZ, Stjernschantz J) Alan R Liss, New York, 1989; 349–368.
14. Bito LZ, Fraga A, Blanco J, Camras CB. Long-term maintenance of reduced intraocular pressure by daily or twice daily topical application of prostaglandins to cat or Rhesus monkey eyes. *Investigative Ophthalmology and Visual Science* 1983; **24**: 312–319.
15. Toris CB, Yablonski ME, Wang YL, Hayashi M. Prostaglandin A_2 increases uveoscleral outflow and trabecular outflow facility in the cat. *Experimental Eye Research* 1995; **61**: 649–657.
16. Poyer JF, Gabelt B, Kaufman PL. The effect of topical $PGF_{2\alpha}$ on uveoscleral outflow and outflow facility in the rabbit eye. *Experimental Eye Research* 1992; **54**: 277–283.
17. Gum GG, Kingsbury S, Whitley RD *et al.* Effect of topical prostaglandin PGA_2, PGA_2 isopropyl ester, and $PGF_{2\alpha}$ isopropyl ester on intraocular pressure in normotensive and glaucomatous canine eyes. *Journal of Ocular Pharmacology* 1991; **7**: 107–116.
18. Groeneboer MC, Hoyng PFJ, Kuizenga A. Prostaglandin $F_{2\alpha}$ isopropyl ester versus Iloprost phenacyl ester in rabbit and Beagle eyes. *Current Eye Research* 1989; **8**: 131–138.
19. Studer ME, Martin CL, Stiles J. Effects of 0.005% latanoprost solution on intraocular pressure in healthy dogs and cats. *American Journal of Veterinary Research* 2000; **61**: 1220–1224.
20. Gelatt KN, MacKay EO. Effect of different dose schedules of latanoprost on intraocular pressure and pupil size in the glaucomatous Beagle. *Veterinary Ophthalmology* 2001; **4**: 283–288.
21. Ofri R, Raz D, Kass PH *et al.* The effect of 0.12% unoprostone isopropyl (rescula) on intraocular pressure in normotensive dogs. *Journal of Veterinary Medical Science* 2000; **62**: 1313–1315.
22. Gelatt KN, MacKay EO. Effect of different dose schedules of bimatoprost on intraocular pressure and pupil size in the glaucomatous Beagle. *Journal of Ocular Pharmacology and Therapeutics* 2002; **18**: 525–534.
23. Hellberg MR, Sallee VL, McLaughlin MA *et al.* Preclinical efficacy of travoprost, a potent and selective FP prostaglandin receptor agonist. *Journal of Ocular Pharmacology and Therapeutics* 2001; **17**: 421–432.
24. Hellberg MR, McLaughlin MA, Sharif NA *et al.* Identification and characterization of the ocular hypotensive efficacy of travoprost, a potent and selective FP prostaglandin receptor agonist, and AL-6598, a DP prostaglandin receptor agonist. *Survey of Ophthalmology* 2002; **47**: S13–S33.
25. Eisenberg DL, Toris CB, Camras CB. Bimatoprost and travoprost; a review of recent studies of two new glaucoma drugs. *Survey of Ophthalmology* 2002; **47**: S105–S115.
26. Goldberg I, Cunha-Vaz J, Jakobsen JE *et al.* Comparison of topical travoprost eye drops given once daily and timolol 0.5% given twice daily in patients with open-angle glaucoma or ocular hypertension. *Journal of Glaucoma* 2001; **10**: 414–422.
27. Orengo-Nania S, Landry T, Von Tress M *et al.* Evaluation of travoprost as adjunctive therapy in patients with uncontrolled intraocular pressure while using timolol 0.5%. *American Journal of Ophthalmology* 2001; **132**: 860–868.
28. Fellman RL, Sullivan EK, Ratliff M *et al.* Comparison of travoprost 0.0015% and 0.004% with timolol 0.5% in patients with elevated intraocular pressure: a 6-month, masked, multicenter study. *Ophthalmology* 2002; **109**: 998–1008.
29. Netland PA, Landry T, Sullivan EK *et al.* Travoprost compared with latanoprost and timolol in patients with open-angle glaucoma or ocular hypertension. *American Journal of Ophthalmology* 2001; **132**: 472–484.
30. SAS/STAT. *Guide for Personal Computers*, Version 6ed, SAS Institute, Inc., Cary, NC.
31. Gelatt KN, Gum GG, Barrie KP, Williams LW. Diurnal variations in intraocular pressure in normotensive and glaucomatous Beagles. *Glaucoma* 1981; **3**: 121–124.
32. Orzalesi N, Rossetti L, Invernizzi T *et al.* Effect of timolol, latanoprost, and dorzolamide on circadian IOP in glaucoma or ocular hypertension. *Investigative Ophthalmology and Visual Science* 2000; **41**: 2566–2573.
33. Yoshitomi T, Ito Y. Effects of indomethacin and prostaglandins on the dog iris sphincter and dilator muscles. *Investigative Ophthalmology and Visual Science* 1988; **29**: 127–132.
34. Lu W, Okamura T, Bian K *et al.* Prostaglandins involved in contractions by angiotension II and bradykinin of isolated dog sphincter pupillae. *British Journal of Pharmacology* 1988; **95**: 544–550.
35. Dziezyc J, Millichamp NJ, Keller CB, Smith WB. Effects of prostaglandin $F_{2\alpha}$ and leukotriene D_4 on pupil size, intraocular pressure, and blood–aqueous barrier in dogs. *American Journal of Veterinary Research* 1992; **53**: 1302–1304.
36. Gwin RM, Gelatt KN, Gum GG *et al.* The effect of topical pilocarpine on intraocular pressure and pupil size in the normotensive and glaucomatous Beagle. *Investigative Ophthalmology and Visual Science* 1977; **16**: 1135–1142.
37. Gum GG, Gelatt KN, Gelatt JK, Jones R. The effect of topical demecarium bromide and echothiophate iodide on intraocular pressure and pupil size in normal beagles and beagles with inherited glaucoma. *American Journal of Veterinary Research* 1993; **54**: 287–293.
38. Gelatt KN, Larocca RD, Gelatt JK *et al.* Evaluation of multiple doses of 4% and 6% timolol and timolol combined with 2% pilocarpine in normotensive and glaucomatous beagles. *American Journal of Veterinary Research* 1995; **56**: 1325–1331.
39. King TC, Gum GG, Gelatt KN. Evaluation of a topical carbonic anhydrase inhibitor (MK-927) in normotensive and glaucomatous beagles. *American Journal of Veterinary Research* 1991; **52**: 2067–2070.