Comparison of two- and three-times-daily topical ophthalmic application of 0.005% latanoprost solution in clinically normal dogs

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OBJECTIVE
To determine whether 2- or 3-times-daily application of topical ophthalmic 0.005% latanoprost solution is more effective at lowering intraocular pressure (IOP) in clinically normal dogs.

ANIMALS
9 clinically normal dogs.

PROCEDURES
For each dog, 1 drop of latanoprost 0.005% solution was applied to 1 eye every 8 or 12 hours each day for 5 days; the contralateral eye received topical ophthalmic treatment with 1 drop of saline (0.9% NaCl) solution at the times of latanoprost application. Ocular examinations of both eyes were performed every 6 hours starting 48 hours prior to and ending 42 hours after the treatment period. Following a 5-week washout interval, the procedures were repeated but the previously latanoprost-treated eye of each dog received latanoprost application at the alternate frequency.

RESULTS
Mean ± SD IOP reduction in the latanoprost-treated eyes was 31 ± 6.9% with 2-times-daily application and 33 ± 8.2% with 3-times-daily application. A 2-way repeated-measures ANOVA revealed significant differences in IOP with contributions by treatment (2 or 3 times daily), time of day (diurnal variation), and individual dog. The maximum mean daily IOP reduction in latanoprost-treated eyes was detected on day 3 of latanoprost treatment in each group. Eyes treated 3 times daily had significantly smaller pupil diameter and greater conjunctival hyperemia than eyes treated 2 times daily.

CONCLUSIONS AND CLINICAL RELEVANCE
The clinical importance of the ocular hypotensive effects of 3-times-daily topical ophthalmic application of 0.005% latanoprost solution in dogs with glaucoma warrants investigation. (Am J Vet Res 2015;76:625–631)

G laucoma is a common cause of irreversible blindness in dogs and has a prevalence of 1.7% in that species. The disease is characterized by progressive vision loss as a result of retinal damage, with elevated IOP being the primary risk factor for development and progression of the condition. Medical treatment for glaucoma typically involves maintaining the IOP at a normal or subnormal level with topically applied ophthalmic medications. Various classes of medication are available for this purpose, each with specific indications, efficacy, and adverse effects. This safety, tolerance, and effects of topically applied prostaglandins on the eye have been reported, and prostaglandin analogs are commonly used to lower IOP in dogs with glaucoma. This effect is achieved by binding of the agents to prostaglandin F receptors within the anterior segment, resulting in increased aqueous humor outflow through the uveoscleral pathway. Latanoprost, a prostaglandin F<sub>2α</sub> analog, is commercially available as a 0.005% solution and has been shown to be effective at lowering IOP in both normal and glaucomatous canine eyes, with a greater effect in glaucomatous eyes. In dogs, 2-times-daily application of the medication reduces the IOP more than once-daily application. In contrast, 2-times-daily application of 0.005% latanoprost solution in human eyes is less effective than once-daily application. Determination of the most effective frequency of treatment with 0.005% latanoprost solution in dogs with glaucoma is important, given that long-term management often exhausts available medication options, resulting in poorly controlled IOP, ocular discomfort, and vision loss. Some veterinary ophthalmologists recommend applying 0.005% latanoprost solution 3 times daily.

ABBREVIATION
IOP Intraocular pressure
times a day in dogs with uncontrolled primary glauco-
ma, especially when multimodal treatment is failing. Al-
though anecdotal evidence may suggest this increased
frequency of treatment is beneficial in some dogs with
glaucoma, there has been no published peer-reviewed
information supporting the recommendation, to our
knowledge.

The primary objective of the study reported here
was to determine whether 2- or 3-times-daily applica-
tion of topical ophthalmic 0.005% latanoprost solu-
tion is more effective at lowering IOP in clinically nor-
mal dogs. Secondary objectives included comparison
of the incidence and severity of adverse effects at the
treatment frequencies.

Materials and Methods

Approval for this study was obtained from the
Iowa State University Institutional Animal Care and
Use Committee. The study was conducted in accor-
dance with the Association for Research in Vision and
Ophthalmology Statement for the Use of Animals in
Ophthalmic and Vision Research.

Nine university-owned clinically normal mixed-
breed dogs with a median age of 2.5 years (range, 1 to 6
years) were enrolled in the study. The group consisted of
4 sexually intact males and 5 sexually intact females that
were housed indoors with a photoperiod of 12 hours
of light and 12 hours of darkness. Two days prior to the
start of the study, findings of ophthalmic examinations,
including Schirmer tear test,1 fluorescein staining,2
tonometry,3 gonioscopy,4 slit-lamp biomicroscopy,5 and
binocular indirect ophthalmoscopy,6 were considered
normal for all dogs.

The 9 dogs were randomly assigned to 1 of 2
groups. One eye of each dog was randomly select-
ed for topical ophthalmic treatment with 1 drop of
0.005% latanoprost solution6 either 2 times (6:00 AM
and 6:00 PM) or 3 times (6:00 AM, 2:00 PM, and 10:00 PM)
daily for a 5-day treatment period. The contralateral
eye of each dog in each group received 1 drop of sa-
lene (0.9% NaCl) solution4 at the times of latanoprost
application. On each day, 6:00 AM was designated as
0 hours. Personnel who provided all the treatments
were not involved in animal examinations.

For all dogs during a 9-day study period, the same
evaluator (KLT) assessed ocular variables 4 times daily
(6:00 AM [before application of latanoprost or saline solu-
tion], noon, 6:00 PM, and midnight) in a blinded fashion.
Pretreatment baseline values were obtained on days 1
and 2 (0 to 48 hours), treatment was provided begin-
ning on day 3 and data were collected during a 5-day
treatment period (days 3 to 7 [48 to 168 hours]), and
follow-up data after discontinuation of treatment were
collected on days 8 and 9 (168 to 210 hours). Evaluated
variables included IOP, pupil diameter, absence or sever-
ity and extent of conjunctival hyperemia, and absence or
severity of aqueous flare. Tonometry was performed in
accordance with the manufacturer’s recommendations.
Horizontal pupil diameter was measured with a Jameson
caliper placed adjacent to the cornea at the level of the
pupil. Conjunctival hyperemia and aqueous flare were
evaluated with a slit-lamp biomicroscope and graded by
means of semiquantitative methods (Appendix).19

Following a 5-week washout period, the proce-
dures were repeated but the previously latanoprost-
treated eye of each dog received latanoprost applica-
tion at the alternate treatment frequency and the
control eye received saline solution application at the
alternate treatment frequency. In this manner, data
for both 2- and 3-times-daily applications of 0.005%
latanoprost solution or saline solution were collected
from the same eye in each dog.

Statistical analysis

Statistical analysis of data was performed with
software.1 Mean ± SD IOP for the different study pe-
riods (baseline, treatment, and follow-up) was cal-
culated. Two-way repeated-measures ANOVA with
Bonferroni posttest was used to compare quantitative
values among baseline, treatment, and follow-up peri-
ods between treatments and between treated and un-
treated eyes. For comparisons among baseline, treat-
ment, and follow-up periods, mean values for each eye
over the period were used. Values of \( P \leq 0.05 \) were con-
sidered significant. The validity of the sample size used
in this project (n = 9) was confirmed with an online
sample size calculator,1 which calculated that a sample
size of 8 would be needed to detect significant differ-
ces with \( \alpha = 0.05 \) and power = 0.80.

Results

IOP

Intraocular pressures for the treated and untreated
eyes during the baseline, treatment, and follow-up peri-
odes were summarized (Table 1). Variation of IOP dur-
ing the pretreatment period was affected by time of day
(\( P = 0.004 \)) and by individual dog (\( P = 0.008 \)) but not
by group assignment (\( P = 0.105 \)). In each treatment fre-
cuency group, mean IOP during the treatment period
was significantly lower in the latanoprost-treated eyes
of the dogs, compared with findings for their contralat-
eral saline-solution–treated eyes (\( P < 0.001 \)). During this
period, IOP values of latanoprost-treated eyes were also
lower than values obtained in the ipsilateral eyes during
the baseline period (\( P = 0.021 \)). When the IOPs of latano-
prost-treated eyes in the baseline and treatment periods
were compared, the mean ± SD reduction during the
treatment period was 31 ± 6.9% for dogs’ eyes treated 2
times daily and 33 ± 8.2% for dogs’ eyes treated 3 times
daily, reflecting a mean IOP reduction of 0.87 mm Hg
more in dogs’ eyes treated 3 times daily. Two-way repeat-
ed-measures ANOVA of each of the 4 daily measurements
of IOP for each dog revealed an effect of treatment (\( P <
0.001 \)) and by individual dog (\( P < 0.001 \)), but no interaction
between treatment and time of day (\( P = 0.400 \)). Subject
matching was effective (\( P < 0.001 \)). Although the differ-
ences in IOP of the treatment groups were significant,
the magnitude was small and unlikely to be clinically
relevant.
Table 1—Mean ± SD IOP (mm Hg) of eyes of 9 clinically normal dogs before, during, and after (baseline, treatment, and follow-up periods, respectively) topical ophthalmic administration of 0.005% latanoprost solution or saline (0.9% NaCl) solution 2 or 3 times daily for 5 days.

<table>
<thead>
<tr>
<th>Period</th>
<th>Latanoprost Solution</th>
<th>Saline solution</th>
<th>Latanoprost solution</th>
<th>Saline solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>14.63 ± 3.43</td>
<td>14.46 ± 3.54</td>
<td>13.79 ± 3.31</td>
<td>13.74 ± 3.47</td>
</tr>
<tr>
<td>Treatment</td>
<td>10.04 ± 2.47</td>
<td>13.34 ± 3.22</td>
<td>9.17 ± 2.52</td>
<td>13.16 ± 3.17</td>
</tr>
<tr>
<td>Follow-up</td>
<td>10.85 ± 2.27</td>
<td>13.24 ± 3.05</td>
<td>10.59 ± 2.30</td>
<td>12.67 ± 2.55</td>
</tr>
</tbody>
</table>

For all eyes, pretreatment baseline values were obtained on days 1 and 2 (0 to 48 hours); treatment for 5 days was provided beginning on day 3, and data were collected on days 3 to 7 (48 to 168 hours). For each dog, 1 drop of latanoprost solution was applied in 1 eye every 12 hours or every 8 hours; 1 drop of saline solution was applied to the contralateral eye at the same times. Follow-up data after discontinuation of treatments were collected for all eyes on days 8 and 9 (168 to 210 hours). On each study day, 6:00 AM was designated as 0 hours; ocular measurements were made by the same investigator 4 times daily (6:00 AM [before application of latanoprost or saline solution], noon, 6:00 PM, and midnight). Following a 5-week washout period, the procedures were repeated but the previously latanoprost-treated eye of each dog received latanoprost application at the alternate treatment frequency and the control eye received saline solution application at the alternate treatment frequency. In this manner, data for both 2- and 3-times-daily applications of 0.005% latanoprost solution or saline solution were collected from the same eye in each dog.

In both treatment frequency groups, the maximum mean daily reduction of IOP in latanoprost-treated eyes was detected on day 3 of the latanoprost treatment period (Figure 1). Maximum mean daily reduction of IOP from baseline in dogs receiving 0.005% latanoprost solution 2 times or 3 times daily was 35% and 40%, respectively. The IOP of latanoprost-treated eyes did not return to baseline values within 42 hours after the last treatment (P = 0.130).

The IOP in eyes that received administration of saline solution decreased during the treatment period, compared with baseline values (Figure 2). Two-way repeated-measures ANOVA of IOP in eyes that were not treated with latanoprost in both phases of the experiment revealed only a subject (matching) contribution to variation (P < 0.001), with no effect of treatment on the contralateral eye (P = 0.072).

Pupil diameter
Mean ± SD pupil diameters for the baseline, treatment, and follow-up periods were summarized (Table 2). In dogs receiving 0.005% latanoprost solution 2 times or 3 times daily, the mean pupil diameter measured during the treatment period was significantly (both P < 0.001) smaller in the latanoprost-treated eyes of each dog, compared with the eyes receiving saline solution as well as the baseline values. Only time of day contributed to variation in pupil size among saline solution–treated control eyes (P < 0.001 for time of day and P = 0.884 for saline solution treatment). During the treatment period, pupil diameter was significantly (both P < 0.001) different between the 2 treatment frequency groups, with contributions by treatment and time of day. Individual dog variation did not contribute to pupil diameter (P = 0.482). In each group, the pupil diameter in latanoprost-treated eyes approached baseline 42 hours after the last administration (Figure 3) but were significantly (P = 0.022) different. The pupil diameter of eyes receiving saline solution significantly (P = 0.008) decreased during the treatment period, compared with baseline values (Figure 4), but differences were not associated with treatment frequency group (P = 0.969) and there was no effect of subject matching (P = 0.996).
Conjunctival hyperemia

In both treatment frequency groups, conjunctival hyperemia during the treatment period was significantly (both \( P < 0.001 \)) greater in the latanoprost-treated eye of each dog, compared with the eye receiving saline solution or baseline values. During the treatment period, the mean \( \pm \) SD conjunctival hyperemia grades for dogs receiving 0.005% latanoprost solution 2 times or 3 times daily were 0.95 \( \pm \) 0.53 and 1.06 \( \pm \) 0.55, respectively. Grade of conjunctival hyperemia in latanoprost-treated eyes was significantly \( (P = 0.013) \) different between the treatment frequency groups. The degree of conjunctival hyperemia in latanoprost-treated eyes was also affected by time of day \( (P = 0.013) \), with less hyperemia at 6:00 AM.

In each treatment frequency group, conjunctival hyperemia in latanoprost-treated eyes was incompletely resolved within 42 hours after the last administration \( (P = 0.018) \), with scores for dogs treated 3 times daily being slightly greater than scores for dogs treated twice daily \( (P = 0.044) \). In either treatment frequency group, the grade of conjunctival hyperemia in the eyes receiving saline solution did not differ \( (P < 0.001) \) from baseline value at any time point.

Aqueous flare

During the treatment period, 2 dogs receiving treatment 3 times daily had aqueous flare of grade 1+ in their latanoprost-treated eye. Aqueous flare was first observed 18 and 24 hours after the first application of latanoprost in these 2 dogs. In each dog, aqueous flare resolved in 12 to 18 hours.

Other variables

No blepharospasm or epiphora developed at any time. Results of fluorescein staining of all eyes were negative at the end of the study.

Discussion

Latanoprost, a prostaglandin F\(_{2\alpha}\) analog, is an ocular hypotensive medication commonly used in dogs and people. Although the mechanism of action is not well studied in dogs, latanoprost predominantly reduces IOP by increasing aqueous humor outflow through the uveoscleral pathway.\(^1,16,17\) In primates, this effect occurs by metalloproteinase-mediated alteration of the extracellular matrix in the ciliary body.\(^20\) Latanoprost may also reduce aqueous humor production and increase trabecular outflow;\(^21,22\) however, the latter mechanism has recently been disputed.\(^17\)

In clinically normal dogs, once-daily and twice-daily administration of 0.005% latanoprost solution reduces IOP by 20% to 40%.\(^2,22-24\) This effect is amplified to 50% to 60% in glaucomatous eyes.\(^1,12\) Although many dogs with primary glaucoma initially respond to latanoprost, elevated IOP and vision loss often develop within 1 year, despite ongoing medical treatment.\(^1\) Surgical intervention with endolaser
Cyclophotocoagulation or combined gonioimplantation and cycloablation may provide the best prognosis for dogs with primary glaucoma to have comfortable eyes with the ability to see. However, many clients decline surgical intervention in favor of medical treatment owing to the surgical costs, guarded long-term prognosis for vision, and inconvenience of travel to a facility with the necessary surgical capabilities. We designed the present study to help guide medical treatment recommendations when surgical intervention is not feasible.

In the clinically normal dogs of the present study, 3-times-daily application of 0.005% latanoprost solution reduced IOP more than did 2-times-daily application (33% and 31%, respectively), which was significant when 2-way repeated-measures ANOVA was performed, permitting assessment of variation associated with treatment frequency group, time of day, and individual dog. Although the differences in IOP of the treatment groups were significant, the magnitude was small and unlikely to be clinically relevant. However, results of previous studies suggest that dogs with glaucoma have greater reduction in IOP than clinically normal dogs when treated with ocular hypotensive medications. Further studies are warranted to determine the degree of IOP reduction that occurs in dogs with glaucoma administered 0.005% latanoprost solution 3 times daily. Pending further research, administration of 0.005% latanoprost solution 3 times daily should be performed cautiously because of the potential for the medication to narrow the iridocorneal angle and to increase episcleral venous pressure, which may impair conventional aqueous humor outflow.

Latanoprost induces miosis by directly stimulating iris sphincter muscle prostaglandin F receptors. Consistent with previous reports, miosis was a common effect of 0.005% latanoprost solution in the dogs used in the present study. Pupil diameter in eyes receiving latanoprost application 3 times daily was significantly smaller than that in eyes receiving latanoprost application 2 times daily, which may increase the risk of pupillary block in some individuals, preclude thorough evaluation of the ocular fundus, and impair vision, particularly at night.

Development of conjunctival hyperemia after topical ophthalmic treatment with various prostaglandin analogs, including latanoprost, has been reported. This conjunctival hyperemia occurs as a result of nitric oxide–mediated vasodilation rather than as a proinflammatory effect, although local irritation may also contribute. In the present study, topical ophthalmic application of 0.005% latanoprost solution 3 times daily resulted in a significantly higher grade of conjunctival hyperemia than did application 2 times daily, although this difference was small and unlikely to be clinically important. Conjunctival hyperemia, although distinct from the episcleral congestion characteristic of glaucoma, complicates interpretation of ocular redness and may reduce sensitivity of an important monitoring variable for increased IOP.

Blood-aqueous barrier disruption associated with latanoprost has been reported and may be related to the role of prostaglandins in uveitis. The blood-aqueous barrier of dogs is labile and relatively susceptible to disruption following topical ophthalmic application of 0.005% latanoprost solution with possible contribution by latanoprost-mediated endogenous...
prostaglandin release. Aqueous flare—indicative of blood-aqueous barrier disruption—developed in 2 of the dogs receiving 3-times-daily administration of 0.005% latanoprost solution in the present study. Because aqueous flare has not been described in most canine studies of once-daily and twice-daily application of 0.005% latanoprost solution, we speculated that application of 0.005% latanoprost solution 3 times daily precipitated the aqueous flare in the 2 dogs in the present study.

Additional adverse effects of topical ophthalmic application of 0.005% latanoprost solution in people include hypertrichosis, eyelash pigmentation, superficial corneal erosions, iris pigmentation, and cystic macular edema. None of these effects were identified in the dogs of the present or previous studies, but protracted treatment courses have not been evaluated in dogs.

Limitations of the present study included the small sample size of clinically normal dogs, which may not accurately reflect the ocular response of dogs with glaucoma to 0.005% latanoprost solution. Furthermore, the pathophysiology of different forms of glaucoma in dogs is complex, which may result in variable therapeutic effects of antiglaucoma medications. Finally, saline solution rather than the latanoprost vehicle (which was not available for use) was used as the control treatment in this study; for this reason, it is unclear whether the drug vehicle contributed to the effects observed in the latanoprost-treated eyes.

Results of the present study indicated that topical ophthalmic application of 0.005% latanoprost solution 2 or 3 times daily resulted in significantly different reductions in IOP in clinically normal dogs, with the higher treatment frequency causing significantly lower IOP and greater miosis and conjunctival hyperemia. Because dogs with glaucoma often have greater IOP reductions than clinically normal dogs when treated with ocular hypotensive medications, additional studies are warranted to determine the effect of application of 0.005% latanoprost solution at a frequency of 3 times daily in glaucomatous canine eyes.

Acknowledgments
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References
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**Appendix**

Semiquantitative grading scales used to assess conjunctival hyperemia and aqueous flare in a study of 9 clinically normal dogs receiving topical ophthalmic treatment with 1 drop of 0.005% latanoprost solution in 1 eye and 1 drop of saline (0.9% NaCl) solution in the contralateral eye 2 or 3 times daily for 5 days.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Grade</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conjunctival hyperemia</td>
<td>0</td>
<td>Normal; blanched to pink</td>
</tr>
<tr>
<td></td>
<td>1+</td>
<td>Flushed red-pink color; affecting predominantly perilimbal bulbar conjunctiva</td>
</tr>
<tr>
<td></td>
<td>2+</td>
<td>Bright red color; affecting most of the bulbar conjunctiva</td>
</tr>
<tr>
<td></td>
<td>3+</td>
<td>Dark red color; affecting both the bulbar and palpebral conjunctiva</td>
</tr>
<tr>
<td>Aqueous flare</td>
<td>0</td>
<td>No flare</td>
</tr>
<tr>
<td></td>
<td>1+</td>
<td>Faint flare (barely detectable)</td>
</tr>
<tr>
<td></td>
<td>2+</td>
<td>Moderate flare (iris and lens detail clear)</td>
</tr>
<tr>
<td></td>
<td>3+</td>
<td>Marked flare (iris and lens detail hazy)</td>
</tr>
<tr>
<td></td>
<td>4+</td>
<td>Intense flare (fixed, coagulated aqueous humor with considerable fibrin)</td>
</tr>
</tbody>
</table>