Effects of Topical 0.5% Levobunolol Alone or in Association With 2% Dorzolamide Compared With a Fixed Combination of 0.5% Timolol and 2% Dorzolamide on Intraocular Pressure and Heart Rate in Dogs Without Glaucoma

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The goal of glaucoma management is to reduce intraocular pressure (IOP) and maintain it at a level compatible with the health of the optic nerve. New therapies are constantly being sought. Topical instillation of levobunolol 0.5%, alone or with dorzolamide 2%, has a hypotensive effect on the IOP in healthy dogs, and levobunolol combined with dorzolamide produces a stronger hypotensive effect than the combination of timolol and dorzolamide. All animals tolerate these topical medications well with no signs of discomfort, and no ocular side effects have been observed. Levobunolol, alone or in combination with dorzolamide, induces bradycardia, as does timolol with dorzolamide.

INTRODUCTION

The term glaucoma refers to a related group of insidious clinical syndromes that occur in humans and domestic animals and are characterized by optic nerve damage associated with a pathologic increase in intraocular pressure (IOP). Glaucoma is a leading cause of vision loss, can lead to globe distortion, and can produce acute and chronic pain, all of which can significantly affect quality of life.1-3 Hence, effective treatments continue to be sought.

The goal of glaucoma management is to reduce IOP and maintain it at a level compatible with the health of the optic nerve. Therapy is aimed at either decreasing the production of aqueous humor or increasing the outflow of aqueous humor from the eye.3,4 Carbonic anhydrase inhibitors and β-adren-
ergic antagonists are two classes of topical drugs that are used most commonly to decrease the production of aqueous humor, and several of these drugs have been evaluated for their effect on the IOP of dogs and cats.\textsuperscript{5–12} Levobunolol is a potent nonselective $\beta$-adrenoceptor blocker that produces a hypotensive effect in humans with glaucoma or ocular hypertension\textsuperscript{13,14}; a recent study has also shown its efficacy in clinically healthy cats.\textsuperscript{15} In the feline study, the authors demonstrated a significant hypotensive effect of levobunolol compared with timolol, another nonselective $\beta$-adrenoceptor blocker, but a synergistic effect in inducing a decline in IOP was not observed when dorzolamide, a carbonic anhydrase inhibitor, was added.\textsuperscript{15}

The objectives of the current study were:

- To examine the effects on IOP of 0.5% levobunolol HCl used topically, both alone and in combination with 2% dorzolamide HCl, in clinically healthy dogs
- To compare the above two protocols with one combining 0.5% timolol maleate with 2% dorzolamide HCl
- To evaluate possible topical adverse effects and/or alteration of heart rate (HR) associated with each protocol

\textbf{MATERIALS AND METHODS}

Ten healthy mixed-breed dogs (five female and five male) were selected for this study with the owners’ informed consent. The age of the animals ranged between 8 and 14 months (median age: 10 months) and their body weight was 14 to 23 kg (median weight: 20 kg).

The selected dogs were determined to be free from ophthalmic pathology by examination using slit-lamp biomicroscopy, indirect ophthalmoscopy, and, after application of topical anesthetic (oxibuprocainehydrochloride), applanation tonometry (Tonopen-XL, Medtronic).

The study was divided into four consecutive periods of 6 days separated by washout periods of 10 days. The same dogs were used throughout the study. In each period, a separate protocol was tested: protocols A, B, and C tested the effects of the drugs, while protocol D evaluated a placebo as the control. All drugs and placebo treatments were applied to both eyes of all the dogs. One drop of 0.5% levobunolol HCl (Vistagan 0.5%, Allergan) was applied in protocol A; one drop of 0.5% levobunolol (Vistagan 0.5%) and one drop of 2% dorzolamide HCl (Trusopt, Merck, Sharp & Dohme) in protocol B; one drop of 0.5% timolol maleate combined with 2% dorzolamide HCl (Cosopt, Merck, Sharp & Dohme) in protocol C; and one drop of artificial tears as placebo (Blu Sal; Sofort, Italy) in protocol D. All treatments were performed by the same observers every 12 hours (8:00 AM and 8:00 PM); in protocol B, the application of levobunolol was followed by the administration of dorzolamide 5 minutes later.

The dogs were housed in a temperature-controlled environment (22°C to 23°C) for 2 days before the start of the research trial and were exposed to an automatic 12-hour light-dark regimen (light phase from 7:00 AM to 7:00 PM, dark phase from 7:00 PM to 7:00 AM).

Each 6-day study period was divided into a pretreatment phase (2 days) and a treatment phase (4 days). During both phases, IOP was measured by applanation tonometry after the administration of a topical anesthetic, and HR was ascertained by a stethoscope placed on the chest over the point of maximum heartbeat intensity. The IOP and HR values were recorded in all animals five times daily (7:00 AM, 10:00 AM, 1:00 PM, 5:00 PM, and 9:00 PM). IOP and HR were measured at the same time points during all phases. All the observers were blinded as to protocol order, and those who administered treatments were different from
those who recorded the IOP and HR. All dogs were monitored for signs of discomfort (e.g., epiphora, conjunctival hyperemia) after the application of drops.

Using the mean value of the IOP of both eyes of each dog, statistical analysis was performed using a two-way repeated measures analysis of variance (ANOVA) test with a Tukey posttest to evaluate the pharmacologic efficacy of treatments, compare the data obtained, and examine the differences in IOP during each day at different times. Lastly, a two-sided paired t test was used to compare the differences between the daily mean values of the pretreatment phase and the last day of the treatment phase, as well as the differences among the daily means.

**RESULTS**

**Pretreatment Phase**

The baseline IOP (mean of the 2 days ± SD) established in this study for each dog during the pretreatment phase of each study period was within the normal range; a diurnal fluctuation in IOP was observed. The mean ± SD IOPs of all dogs for each protocol were not significantly different (P > .05). The mean of the 2 days ± SD HR of all dogs for each protocol also showed no significant differences (P > .05). Because no significant difference in IOP and HR values between dogs was detected during the pretreatment period, baseline values were not included as covariates, and statistical testing occurred only between treatments.

**Treatment Phase: Intraocular Pressure**

All of the animals tolerated the topical medications well with no signs of discomfort, and no ocular adverse effects were observed. Results are reported in Figure 1.

On the first day of treatment, the mean values ± SD of IOP in protocols A, B, C, and D were 15.77 ± 2.72, 14.95 ± 3.03, 15.87 ± 3.10, and 18.17 ± 2.44 mm Hg, respectively. There were no significant differences among treatments, but there were significant differences between the treatment protocols and placebo protocol D.

In detail, 2 hours after the first application of drops, all three experimental protocols (A, B, and C) resulted in reduced IOP; 5 hours after the first application, there was a significant difference between protocols A and B (17.76%) (P < .01) and between B and C (15.65%) (P < .01), with a stronger hypotensive effect from protocol B.

On the second and the third days of treatment with two experimental protocols (B and C), comparison of mean daily values of IOP with mean values from the previous day of each protocol showed a significant decrease in

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![Figure 1](image.png)

**Figure 1.** This graph illustrates the variation in mean IOP values during the pretreatment and treatment phases, showing the stronger IOP-lowering effect of protocol B compared with the other protocols.
IOP. Protocol A showed a significant decrease only on day 3 compared with day 2. During these 2 days, there were significant differences only between the mean values obtained with protocols A and B and between those obtained with A and C, with a weaker hypotensive effect from protocol A (Figure 1).

On the fourth day of treatment, the mean values ± SD of IOP were 13.72 ± 1.20, 10.92 ± 0.97, 12.25 ± 1.05, and 18.50 ± 1.50 mm Hg for protocols A, B, C, and D, respectively, with significant differences between protocols. Compared with the mean values of IOP obtained on the previous day, there were significant differences for protocols A and B of 5.83% (P < .05) and 9.53% (P < .001), respectively, but not for protocol C (P > .05), with the greatest hypotensive effect shown by protocol B. There was a difference of 2.80 mm Hg (P < .001) between protocols A and B and a difference of 1.323 mm Hg (P < .001) between protocols B and C. Comparison of the data from the last day obtained in the three experimental protocols (A, B, and C) with those of the control protocol (D) showed significant differences for all treatments.

**Treatment Phase: Heart Rate**

The mean values ± SD of HR during the first day of treatment were 93.80 ± 20.70, 87.50 ± 21.00, 75.50 ± 14.88, and 96.00 ± 9.62 for protocols A, B, C, and D, respectively. When the values obtained using the three treatment protocols (A, B, and C) were compared with those of the placebo (D), only protocol C caused a significant decrease in HR of 21.35% (P < .001), but there were no significant differences between C and B and between C and A. Results are reported in Figure 2.

On the second day of treatment, all three treatment protocols (A, B, and C) had brought about a significant decrease in HR (P < .001) compared with the mean values obtained for the placebo protocol (D). On the third day of treatment, there was a significant difference between protocols A and D and between C and D (P < .05), but not between protocols B and D (P > .05); there were no significant differences among the three treatment protocols.

On the fourth day of treatment, the mean values ± SD of HR were 71.60 ± 9.16, 83.00 ± 16.25, 75 ± 10.77, and 82.20 ± 8.82 bpm for protocols A, B, C, and D, respectively. Compared with the daily mean values of HR, only protocol A was associated with a significant decrease in HR of 12.83% (P < .05) compared with the control protocol D, but there were no significant differences among the three treatment protocols.

Comparison of the last day of the treatment phase with the pretreatment phase showed that
mean HR decreased significantly in dogs given all protocols ($P < .0001$); the decrease of HR was 34.37%, 21.99%, 27.04%, and 20.50% in dogs given protocols A, B, C, and D, respectively.

**DISCUSSION**

To our knowledge, the effects of topical levobunolol on IOP have not yet been demonstrated in dogs. However, topical levobunolol produces a statistically significant lowering of IOP in healthy cats.\(^{15}\)

Evaluation of the results obtained in this study with the three pharmacologic protocols compared with placebo showed that the protocols containing levobunolol caused a reduction in IOP in healthy dogs, but only the levobunolol–dorzolamide protocol gave significant results compared with the combination of timolol and dorzolamide.

Two hours after the first application of the drops, all three experimental protocols (A, B, and C) resulted in a significant reduction in IOP, with the maximum effect occurring 2 to 5 hours after the first application of the levobunolol–dorzolamide combination. This finding is comparable with the peak reduction at 2 to 7 hours that is observed with other topical β-blockers in humans, rabbits, and dogs.\(^{4,17-19}\) The results presented here showed a significant drop in IOP values on the fourth day of treatment compared with those on the third day for protocols A (levobunolol alone) and B (levobunolol–dorzolamide combination) only. These data suggest that use of levobunolol could be advantageous in the treatment of canine glaucoma because it may permit further lowering of the IOP beyond the plateau phase of the third day seen with β-blockers.\(^{4}\)

With respect to side effects, at the end of the study all of the animals had tolerated the eye-drops well. No signs of conjunctival hyperemia or epiphora were observed.

The HR values showed that timolol caused significant bradycardia on the first day of treatment, whereas levobunolol produced bradycardia on the second day. Indeed, although the mean HR values for β-blockers in this study did not differ significantly compared with those for the control protocol, the bradycardic effects of β-blockers must be taken into consideration for animals with heart disease. Lastly, analysis of the HR data obtained from the pretreatment–posttreatment comparison shows that with all the protocols, including the control, the differences between the groups disappeared due to a drop in HR even in the placebo group. This drop was probably a result of an attenuation of sympathetic reply as adaptation to the experimental conditions.

This study did have some limitations. One drawback concerns the crossover design of the study; this has the advantages of a smaller variability and a smaller number of subjects compared with a parallel design, but the weakness of a potential overlap of treatments due to an inadequate washout period. However, this same study design has been used in clinical trials with glaucomatous dogs and is reasonable considering the short duration of the pharmacologic activity of topical β-blockers.\(^{13}\)

The second drawback is that, although the pharmacologic activity is statistically significant, the relevance to the clinical situation of reducing the IOP in dogs with glaucoma is unknown. Therefore, clinical studies with glaucomatous dogs are required to evaluate the hypotensive effects of levobunolol and examine possible topical and systemic adverse effects over a therapeutic interval. Finally, clinical studies should evaluate whether levobunolol can be administered every 12 hours at a dose of 0.5% or less (like timolol).

**CONCLUSION**

Topical instillation of levobunolol 0.5%
alone or in combination with dorzolamide 2% has a hypotensive effect on the IOP of normal dogs. These results suggest that levobunolol in combination with dorzolamide produces a more significant hypotensive effect than the combination of timolol and dorzolamide. Regarding pharmacologic effects, analysis of the data measured at the various time points shows that the levobunolol protocols (alone or in association with dorzolamide) not only caused the quickest drop in IOP but also maintained this hypotensive effect for the longest duration. Lastly, levobunolol—either alone or associated with dorzolamide—induces bradycardia, as does timolol with dorzolamide.

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**REFERENCES**