Effect of topical administration of 2% dorzolamide hydrochloride or 2% dorzolamide hydrochloride-0.5% timolol maleate on intraocular pressure in clinically normal horses

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Objective—To evaluate the effect of topical administration of 2% dorzolamide hydrochloride or 2% dorzolamide hydrochloride-0.5% timolol maleate on intraocular pressure (IOP) in clinically normal horses.

Animals—18 healthy adult horses without ocular abnormalities.

Procedure—The IOP was measured at 5 time points (7 AM, 9 AM, 11 AM, 3 PM, 7 PM) over 11 days. On days 1 and 2, baseline values were established. On days 3 through 5, horses received 2% dorzolamide HCl (group D, n = 9) or 2% dorzolamide HCl-0.5% timolol maleate (group DT, 9) in 1 randomly assigned eye every 24 hours immediately following each daily 7 AM IOP measurement. On days 6 through 9, each drug was given every 12 hours (7 AM and 7 PM) in the treated eye. Measurements on days 10 and 11 assessed return to baseline. Mixed linear regression models compared mean IOP difference for each drug at each time period.

Results—Mean IOP decreased significantly in all eyes during the 2 dose/d period, compared with the baseline, 1 dose/d, and follow-up periods.

Conclusions and Clinical Relevance—Administration of either drug every 24 hours for short-term treatment does not reduce IOP significantly. Administering either drug every 12 hours induced a significant reduction of IOP; however, controlling for all variables, the reduction was less than 2 mm Hg. (Am J Vet Res 2001;61:709–713)

Glaucma results from an alteration in aqueous humor dynamics that causes an increase in intraocular pressure (IOP) above pressures compatible with normal ocular function. Glaucma increasingly is being identified in horses. Veterinarians have more awareness of the characteristic appearance of a glaucomatous eye and have access to portable tonometers that can more accurately document an increase in IOP. Glaucma in horses may develop secondary to inflamm-
normal adult horses in 1 study but failed to significantly reduce IOP in another study population of clinically normal horses. Topical administration of a β-blocker, 0.5% timolol maleate, decreased IOP by 17% in horses with normal eyes, compared with pretreatment values, when 1 dose was administered bilaterally. Application of 0.5% timolol maleate every 12 hours resulted in IOP decreasing 27% from baseline values by the fifth treatment day. The effect of timolol and atropine in clinical cases of horses with glaucoma, however, is reportedly unpredictable.

The CAI reduce IOP by suppressing carbonic anhydrase (CA) activity in the ciliary epithelium, thus reducing aqueous humor production. Systemically administered CAI have been used extensively in the management of humans and dogs with glaucoma. Systemic adverse effects, however, often limit their use in these species, and topical formulations have become available only recently. A CAI formulated for topical use, dorzolamide, has proven effective in decreasing IOP in normal and glaucomatous eyes in people. Application of 2% dorzolamide significantly decreases IOP in horses (4 mares and 5 geldings; group DT) received 0.25 ml of 2% dorzolamide hydrochloride-0.5% timolol maleate in 1 randomly assigned eye. The concentration of dorzolamide was equivalent in both preparations (22.3 mg/ml). The IOP measurements then were performed as previously outlined. This 3-day period was designated period 1 (1 dose/d; IOP measurements with once-daily administration of a drug). On days 6 through 9, the procedure was repeated; however, an additional dose of drug was administered at 7 PM; the appropriate drug was administered in the same assigned eye in both groups. The IOP measurements were obtained at the same 5 time points. This 3-day period was designated period 2 (2 dose/d; IOP measurements with twice-daily administration of a drug). On days 10 and 11, drugs were not administered, but IOP measurements were obtained at the same 5 time points. This period (period 3: follow-up monitoring) was used to establish a return to baseline values.

Data analysis—For each time point, IOP used for analysis was the mean of the 3 tonometry readings for that eye. Mixed linear regression models were used to compare IOP on the basis of eye (treated and untreated), drug (D or DT), or sex for each time period (0, 1, 2, and 3), using the following equation:

\[
Y_{ij} = (\beta_0 + \beta_1 \text{ Eye}) + (\beta_2 \times \text{ Sex}) + (\beta_3 \times \text{ Drug}) + (\beta_4 \times \text{ Time}) + \epsilon
\]

where \(\beta_0\) is the intercept of this regression line for the jth time period (j = 0 to 3), \(\beta_1\) is the mean difference in IOP between the treated and control eyes for the jth time period, \(\beta_2\) is the mean difference in IOP between the drugs for the jth time period, \(\beta_3\) is the mean difference in IOP between the 2 sexes for the jth time period, \(\beta_4\) indicates the slope of the regression line for the jth time period (mean change in IOP for each hour change in time), and \(\epsilon\) is normal random variables. The purpose of the study reported here was to evaluate the efficacy of these products for decreasing IOP in eyes of clinically normal horses.

Materials and Methods

Animals—Eighteen adult horses (8 geldings and 10 mares) were selected as the sample population. Horses were selected on the basis that ocular surface or intraocular abnormalities were not detected during examination by use of biomicroscopy and indirect ophthalmoscopy. Another criterion for selection was appropriate demeanor and tolerance to handling of the head and periocular area. Horses were acclimated to a stall environment for 48 hours prior to initiation of the study. During that time, horses were handled multiple times to familiarize them with restraint techniques required for the subsequent procedures.

Measurement of IOP—Anesthetic (0.5% proparacaine) was applied topically to the corneal surface. Then, IOP was measured in both eyes, using an applanation tonometer. Measurements were obtained 5 times daily (7, 9, and 11 AM and 3 and 7 PM) for 2 days to establish normal variation in IOP during a 12-hour period. The first day on which measurements were obtained was designated day 1, and the 2-day period was designated period 0 (baseline; IOP without administration of any drugs). The same observer performed all measurements, and the tonometer used was within current calibration specifications of the manufacturer. The tonometer was calibrated daily throughout the course of the study. All horses tolerated gentle manual restraint for IOP measurements; therefore, sedation or chemical paralysis of the auriculopalpebral nerve was not required. Three readings were obtained from each eye in sequence. Each reading was a mean of several (4 to 6) corneal applanations having a variance of ≤ 5%.

Drug administration—Immediately after the IOP measurement was obtained at 7 AM on days 3 through 5, 0.25 ml of 2% dorzolamide hydrochloride was instilled in the inferior conjunctival cul-de-sac of 1 randomly assigned eye of each of 9 horses (6 mares and 3 geldings; group D). The remaining 9 horses (4 mares and 5 geldings; group DT) received 0.25 ml of 2% dorzolamide hydrochloride-0.5% timolol maleate in 1 randomly assigned eye. The concentration of timolol maleate was equivalent in both preparations (22.3 mg/ml). The IOP measurements then were performed as previously outlined. This 3-day period was designated period 1 (1 dose/d; IOP measurements with once-daily administration of a drug). On days 6 through 9, the procedure was repeated; however, 1 additional dose of drug was administered at 7 PM; the appropriate drug was administered in the same assigned eye in both groups. The IOP measurements were obtained at the same 5 time points. This 3-day period was designated period 2 (2 dose/d; IOP measurements with twice-daily administration of a drug). On days 10 and 11, drugs were not administered, but IOP measurements were obtained at the same 5 time points. This period (period 3: follow-up monitoring) was used to establish a return to baseline values.

Results

Fit of mixed linear regression models for each of the time periods was determined. Analysis of these models indicated that there was not a significant difference in IOP between treated and untreated eyes or between the 2 drugs during the treatment periods. We did not detect a significant effect of sex during the baseline and treatment periods; however, during the follow-up period, there was a significant effect of sex (P = 0.037). For the
ment (baseline), during once-daily treatment (1 dose/d), or after dorzolamide-0.5% timolol twice daily with periods prior to treatment during which they were treated topically with 2% dorzolamide or 2% dorzolamide-0.5% timolol twice daily with periods prior to treatment (baseline), during once-daily treatment (1 dose/d), or after treatment (follow-up monitoring).

Table 1—Results of a mixed linear regression model that compared intraocular pressure in 18 horses during the period in which they were treated topically with 2% dorzolamide or 2% dorzolamide-0.5% timolol twice daily with periods prior to treatment (baseline), during once-daily treatment (1 dose/d), or after treatment (follow-up monitoring).

<table>
<thead>
<tr>
<th>Variable</th>
<th>95% Confidence limits</th>
<th>Coefficient</th>
<th>P</th>
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<td>Constant Period</td>
<td>17.38, 20.18</td>
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<td>1 dose/d</td>
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<td>Follow-up monitoring</td>
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</tr>
<tr>
<td>Sex</td>
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<td>-1.029</td>
<td>0.999</td>
</tr>
</tbody>
</table>

2 time periods in which drugs were administered (1 and 2), there was a significant (P < 0.001) effect of time. During period 1 (ie, 1 dose/d), mean IOP increased by 0.035 mm Hg for each 1-hour interval after initiation (0.84 mm Hg/d). During period 2 (ie, 2 dose/d), mean IOP decreased by 0.02 mm Hg for each 1-hour interval after initiation (0.48 mm Hg/d).

Mean ± SEM IOP was 19.71 ± 0.45 mm Hg. For all horses, there was not a significant difference in IOP among time points during period 0 on the basis of eye, sex, or drug. This does not address the question of whether a diurnal effect of IOP was evident in horses, because it is still possible that there was substantial variation during the remainder of the 24-hour period when IOP was not measured. However, for the hours during which we evaluated drug effect on IOP in this study (7 AM to 7 PM), we did not detect a significant variation in mean IOP between any time points in horses in either drug group. The reference range of IOP in horses seems to be higher than many other species. Values reported (mean ± SD IOP) for horses include 23.5 ± 6.10 mm Hg,19 20.6 ± 4.7 mm Hg,20 24.5 ± 4.0 mm Hg,21 28.6 ± 4.84 mm Hg,22 27 ± 8.8 mm Hg,23 and 22 to 28 mm Hg.24 Our data are comparable to results reported in those studies.

Figure 1—Graph depicting mean intraocular pressure (IOP) over time for 18 horses that received topical administration of 2% dorzolamide or 2% dorzolamide-0.5% timolol once or twice daily. Points represent mean IOP for horses at each measurement time point. Hours 0 to 3 represent baseline values, hours 48 to 108 represent the 1 dose/d period, hours 120 to 204 represent the 2 doses/d period, and hours 216 to 252 represent the follow-up monitoring period. Although a single time point could not be identified for the peak effect of IOP reduction during treatment periods, a line of best fit through the data points for each period indicated an overall reduction of IOP during period 2.

Discussion

Mean ± SEM IOP for the baseline period was 19.71 ± 0.45 mm Hg. For all horses, there was not a significant difference in IOP among time points during period 0 on the basis of eye, treated or untreated, drug administered, or sex.

Values for overall mean IOP for each time point were plotted (Fig 1). Peak IOP was routinely evident at 7 PM, but the lowest daily values of IOP varied, without a readily definable pattern. Although 1 time point could not consistently be identified for the peak effect of IOP reduction during the periods of administration, a line of best fit through the data points for each period indicated an overall reduction of IOP during period 2.

Administration of dorzolamide or dorzolamide-timolol every 24 hours did not reduce mean IOP from baseline values in treated or untreated eyes. On the contrary, IOP increased in all eyes during period 1. This result was consistent in all horses. Reasons for this increase in mean IOP remain speculative. It may have been the result of the drug wearing off during the day and a rebound effect in which IOP transiently increases in response to lack of the drug. This effect again was suggested following discontinuation of drug administration, at which time a significant effect of sex was identified. The reason for the difference in follow-up mean IOP in mares and geldings was speculated to be associated with a higher rate of change in IOP during the follow-up period in mares as compared to geldings. This effect may have been magnified as a result of the greater number of mares (n = 10) versus geldings (8) in the study population. The peak effect of 2% dorzolamide in humans is observed 2 hours after topical installation, and a significant reduction persists for 8 to 12 hours; however, rebound increases in IOP above baseline values after discontinuation of the drug are not evident.21,22 Mean IOP decreased significantly in all eyes during period 2 in all horses, compared with baseline values and values for period 1. This decrease was not dramatic, equating to a mean decrease in IOP of < 2 mm Hg, compared with baseline values.

In humans, there are at least 7 CA isoenzymes, and 2 of them have been identified in the ciliary epithelium (cytoplasmic CA II and membrane-bound CA IV).25 Carboxylic anhydrase II plays a critical role in the production of aqueous humor in humans, rabbits, and
rate of aqueous humor formation. The effect of topical application of CAI to decrease IOP may be related to poor ocular penetration or incomplete enzyme inhibition. Furthermore, it has been reported that approximately 99% of the enzyme activity must be inhibited to result in a decrease in aqueous humor production. Failure of a topically applied CAI to decrease IOP may be related to poor ocular penetration or incomplete enzyme inhibition. Because early topical formulations of CAI had poor intraocular penetration, the amount of CAI at the ciliary body did not reach a sufficient concentration to suppress aqueous production. To the authors' knowledge, CA isoenzymes have not been isolated from the eyes of horses. Dorzolamide may cause less complete inhibition of CA isoenzymes involved in aqueous production in the eyes of horses, or the target tissue may not adequately absorb the 2% concentration of the drug. In eyes of horses, surface absorption and tissue binding characteristics as well as time of residence at active sites in the ciliary epithelium for 2% dorzolamide currently are unknown. Additionally, it is possible that there are other factors contributing to the production of aqueous humor in eyes of horses, and CAI are not major suppressors of aqueous production in this species. From a therapeutic standpoint, it seems unlikely that the IOP reduction observed in the normal eyes of horses reported here would equate to adequate IOP suppression in a glaucomatous eye of a horse. However, in humans, the IOP-decreasing effect of dorzolamide was greater in glaucomatous eyes than in normotensive eyes. Another topical CAI, MK-927, produced a greater reduction in IOP in glaucomatous eyes of dogs than it did in normotensive eyes of dogs. There is evidence that control of IOP in humans is improved with dosing of 2% dorzolamide 3 times a day, compared with dosing 2 times a day. Although administration of either drug at 8-hour intervals was not evaluated in our study, this could improve efficacy of dorzolamide and dorzolamide-timolol in horses.

The question remains as to whether dorzolamide-timolol has a clinical advantage over either of the drugs administered alone. Timolol is a nonspecific β-adrenergic receptor antagonist that reduces IOP by decreasing the rate of aqueous humor formation. The effect of topical application of timolol on IOP has been evaluated in female horses with normotensive eyes. The concentration of 0.5% timolol used in our study was comparable to that used by van der Woerd et al. Although a decrease in IOP was attributed to timolol in that study, direct comparison of our data with that study's data is inappropriate, because those investigators administered drugs to both eyes, measured IOP at different time points, and evaluated their data with another method of statistical analysis. In a study of humans with increased IOP, the addition of twice-daily administration of 2% dorzolamide to 0.5% timolol decreased IOP by 17% at the morning trough and 19% at peak concentrations. In humans, dorzolamide is a much weaker suppressor of aqueous flow and IOP than timolol, but the 2 drugs in combination have an additive effect that is greater than the effect of either drug used alone. Analysis of results of our statistical model, which controlled for the variables of sex, eye, and time, suggests that there is not a significant difference between the effect of dorzolamide administered alone or in combination with timolol and raises the question of whether timolol truly has an IOP-decreasing effect in the eyes of horses. This question may have been better addressed in the study reported here by evaluating the effect of timolol independently from the effect of dorzolamide-timolol.

An effect on IOP parallel to that observed in treated eyes was evident in untreated eyes of horses in both treatment groups. A decrease of IOP in the contralateral eye in subjects given 0.5% timolol in 1 eye has been documented in dogs, cats, and humans. This effect is likely the result of systemic uptake and a cross-over effect rather than a central effect. For a drug to have an effect in the contralateral eye, it must be absorbed systemically, resulting in effective blood concentrations. Evaluation of blood concentrations of these drugs was beyond the scope of our study. In retrospect, however, monitoring heart rate before and after administration of dorzolamide-timolol may have enabled us to detect changes associated with timolol-related β-blockade. Dorzolamide is systemically absorbed to some degree after topical administration in humans. In humans with glaucoma and other ocular hypertensive disorders that were treated with 2% dorzolamide every 8 hours, RBC concentrations of dorzolamide were detectable, and total CA activity in RBC was reduced by 50% on day 15 of treatment. Dorzolamide also was detected in the urine of these patients. It is plausible that the reduction in IOP observed in the untreated eyes of the horses receiving dorzolamide unilaterally in the study reported here was associated with systemic absorption of the drug, although this was not confirmed by evaluation of blood or urine concentrations. Other overt systemic effects of the drug were not observed in dorzolamide-treated horses.

The fact that the horses in the study reported here had normotensive eyes presents a major limitation to interpretation of results. Additionally, the duration of drug administration was brief. On the basis of our analysis, it appears the dorzolamide-timolol combination may not have additional IOP-decreasing effects over dorzolamide alone that would warrant its use in horses with glaucoma during short-term treatment. Concomitant use of 0.5% timolol and 2% dorzolamide every 12 hours in humans with glaucoma has an IOP-decreasing effect equivalent to that for 2% dorzolamide administered alone every 8 hours. Whether either drug would provide a sustained IOP-decreasing effect in glaucomatous eyes of horses when administered for long-term treatment or at higher dosing frequencies remains speculative. On the basis of analysis of data reported here, topical administration of dorzolamide or dorzolamide-timolol every 12 hours currently can only be recommended as adjunctive treatment for horses with glaucoma. Controlled studies on the long-term effect of these drugs and alternative treatments on glaucomatous eyes of horses are warranted.

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References