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Effect of 0.2% brimonidine and 0.2%/0.5% brimonidine-timolol on intraocular pressure and pupil size in normal equine eyes

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Summary

Background—Brimonidine is an alpha 2-adrenergic agonist that decreases aqueous humor production and may increase uveoscleral outflow. It has not been evaluated in normal or glaucomatous equine eyes.

Objectives—To evaluate the IOP-lowering efficacy and safety of brimonidine, alone and in conjunction with timolol, as a treatment for equine glaucoma by comparing IOP in normal equine eyes treated with brimonidine and brimonidine-timolol to control eyes.

Study design—A balanced crossover design with 16 horses receiving one of two treatments, brimonidine and brimonidine-timolol, during each of two 10-day study phases, was used. Four horses were randomly assigned to each of four combinations of treated eye (right or left) and drug order within the two 10-day study phases (brimonidine first or brimonidine-timolol first).

Methods—Pupil size and conjunctival hyperemia were assessed twice daily and IOP was measured three times daily using rebound tonometry in both eyes of 16 normal horses throughout two 10-day study periods (brimonidine and brimonidine-timolol) followed by an 18-day washout period. One eye of each horse was treated with brimonidine or brimonidine-timolol while the opposite eye was treated with balanced salt solution.

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Authors' declaration of interests

None declared.

Ethical animal research

Research reported in this manuscript followed ARRIVE guidelines, was approved by University of California-Davis Institutional Animal Care and Use Committee, and was performed according to the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Visual Research.

Authorship

M. Von Zup contributed to study design, study execution by performing all measurements during data collection, and data interpretation. M. Lassaline contributed to study design, study execution by examining horses at baseline and following study conclusion, and data interpretation. P. Kass contributed to study design and data analysis and interpretation. P. Miller contributed to study design, and data interpretation. S. Thomasy contributed to study design, study execution by examining horses at baseline and following study conclusion, and data interpretation. All authors contributed to manuscript preparation and gave their final approval of the manuscript.

Results—There were no adverse effects and no significant changes in pupil size in normal equine eyes treated with brimonidine or brimonidine-timolol. Average IOP in normal equine eyes treated with brimonidine (25.6 mm Hg) was statistically higher than in eyes treated with brimonidine-timolol (24.6 mm Hg) or balanced salt solution (24.5 mm Hg) however IOP differences were 1 mm Hg or less and thus not clinically important.

Main limitations—Horses with normal eyes may not be as sensitive to IOP-lowering effects of treatment as horses with glaucoma.

Conclusions—Brimonidine and briminonidine-timolol are well tolerated in normal horses but do not decrease IOP.

Keywords

horse; glaucoma; intraocular pressure; brimonidine; timolol

Introduction

Glaucoma is a devastating disease associated with irreversible degeneration of the optic nerve, which may lead to blindness in horses. Although the specific mechanisms contributing to the initiation and progression of most cases of glaucoma are unknown, the primary risk factor is increased intraocular pressure (IOP). Increased IOP is caused by an imbalance in production and outflow of aqueous humor. In horses, elevated IOP usually occurs due to decreased outflow of aqueous humor which is usually secondary to uveitis [1–3]. In eyes with uveitis, there is an infiltration of inflammatory cells and damage to the trabecular meshwork, which makes it more difficult for aqueous humor to exit the eye.

There are only a few topical ophthalmic medications that have been proven to be effective in decreasing IOP, and therefore presumptively managing glaucoma, in horses. All of these compounds lower IOP by reducing aqueous humor production and include the carbonic anhydrase inhibitors dorzolamide, brinzolamide, and β-blocker timolol [1–3]. Brinzolamide was found to lower IOP by an average of 14–21% in normal horses, depending on dosing once or twice daily [1]. Dorzolamide-timolol was found to lower IOP by an average of 13% when measured in clinically normal horses [4]. Timolol decreased IOP by on average 17% in clinically normal horses [5]. These are relatively small changes in IOP and in the authors' experience, these medications are not effective for all horses with glaucoma. In addition, several glaucoma medications, including latanoprost and pilocarpine, that are effective in human or canine patients are either ineffective or irritating to horses [6, 7, 8]. Thus, there is a need for more effective therapeutic options for glaucoma in horses.

Brimonidine is a commercially available α2-adrenergic receptor agonist that is approved by the Food and Drug Administration for the treatment of glaucoma in humans [9]. The α2-adrenergic receptor agonists decrease IOP by inhibiting adenylate cyclase causing decreased cAMP, which is necessary for production of aqueous humor. Xylazine, a systemic α2-adrenergic receptor, has been shown to reduce IOP by 20% in horses [10]. In addition to decreasing production, brimonidine may increase aqueous humor outflow via the uveoscleral pathway in which fluid exits the eye by diffusing through the ciliary body and

sclera [9, 10, 11]. Horses are unique in that they rely more heavily on uveoscleral outflow in comparison to most species [2, 3]. Therefore, brimonidine may decrease IOP by several different mechanisms in horses.

Timolol is a beta-adrenergic receptor antagonist that reduces production of aqueous humor and is effective at decreasing IOP in normal horses [10]. While brimonidine and timolol administered separately to human patients with glaucoma reduced IOP about the same amount [12], fixed combinations of brimonidine and timolol have been shown to have decreased IOP further than either drug administered alone [9, 11, 13]. In addition, trials with human patients with glaucoma demonstrated that brimonidine alone had more adverse reactions than timolol alone. However, combining medications reduced adverse reactions to less than that of brimonidine alone [11]. Thus, we are interested in investigating the effects of both brimonidine and a fixed combination of brimonidine and timolol on IOP in normal horses. Therefore, the objectives of this study are to evaluate the IOP-lowering efficacy and safety of brimonidine, alone and in conjunction with timolol, in normal equine eyes.

Materials and methods

Animals

Sixteen horses, ten geldings and six mares, were used in this study with a median (range) age of 11.5 (8–18) years. The breeds included were thirteen Thoroughbreds, two Warmbloods, and one Quarter Horse. Prior to initiation of the study, each horse received a thorough ophthalmic exam performed by one of two boarded veterinary ophthalmologists and was confirmed to be free of ocular disease. Specifically, handheld slit lamp biomicroscopy (SL-15[a]) and indirect ophthalmoscopy with a 2.2 D lens[b] and a binocular indirect ophthalmoloscope[c] was performed. Each horse was housed in a covered pen during the study periods and in a paddock during recovery periods. All horses received their usual diet throughout the study periods.

Study design and medication administration

This study was approved by an institutional animal care and use committee and performed according to the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Visual Research. Sixteen horses were used in a balanced crossover design, with eight horses each receiving each of the two treatments, 0.2 ml of 0.2% brimonidine tartrate ophthalmic solution and 0.2% brimonidine tartrate/0.5% timolol maleate ophthalmic solution during either phase I or phase II. A 1 ml syringe with a 25 G ¾ inch needle was filled with each the medication or placebo (balanced salt solution, BSS). The needle was broken off from the hub and the medication was squirted into the eye (12). Horses were randomly assigned to treatment order, such that each horse received one of the two treatments, either brimonidine or brimonidine-timolol, in one randomly selected eye and 0.2 ml of BSS in the opposite eye every 12 hours (at 07.00 h and 19.00 h) for five days during phase I, and then after an 18-day washout period, received the other treatment every

[a]Kowa American Corporation, Torrance, California, USA.
[b]Volk Optical Inc., Mentor, Ohio, USA.
[c]Keller Instruments Inc., Broomall, Pennsylvania, USA.

12 hours for five days during phase II. During each of the two 10-day study periods, baseline measurements were obtained on Days 1–3, measurements were continued and treatment was given on Days 4–8, and measurements were continued post treatment on Days 9 and 10. Fourteen horses completed both treatment trials while two horses completed only a single trial, being removed from the study at the conclusion of phase I after they became refractory to having medications administered and IOP measurements performed. One of these horses had completed phase I in the brimonidine treatment group, and the other horse had completed phase I in the brimonidine-timolol treatment group.

Acclimation and measurement of IOP, pupil size and conjunctival hyperemia

Four days prior to the beginning of Study Period 1, horses were moved into the pens where the trial was conducted in order to begin the acclimation period. During the acclimation period, a single evaluator measured IOP using rebound tonometry (Icare Tono Vet[d]) on the horses twice daily to acclimate them to the procedure. Positive reinforcement with horse treats (Manna Pro Bite Size Nuggets[e]) was used to train the horses to stand calmly for the measurement of IOP. Intraocular pressure was measured until consistent readings were received from each horse and they were no longer reactive to measurement. All of the horses acclimated well over the four-day period and we were able to use positive reinforcement to obtain accurate measurements on the horses without sedation or topical anesthetics.

During the study period, IOP was measured by the same single evaluator masked to treatment three times daily (07.00 h, 13.00 h and 19.00 h). The measurements were taken with the horses standing calmly in a shaded portion of the covered pen with the head maintained in an appropriate position (i.e. not below the heart) [13]. No topical anaesthetics, systemic sedatives or local nerve blocks were used to obtain measurements. At each time point, three IOP readings were recorded. As per the Icare TonoVet manual, each IOP reading represents an average calculated by the rebound tonometer of four out of six IOP measurements, with the highest and lowest measurement of the six excluded. The IOP reading also includes a categorical measure of variability, with “no bar” readings having a standard deviation less than 1.0 mmHg, “low bar” readings having a standard deviation between 1.8 mmHg and 2.5 mmHg, “medium bar” readings having a standard deviation of between 2.5 mmHg and 3.5 mmHg, and “high bar” readings having a standard deviation greater than 3.5 mmHg. At each time point, IOP measurements were continued until three IOP readings that were classified by the tonometer as having no bar, a low bar or a medium bar were obtained. These three IOP readings were recorded; no IOP readings with a high bar were included in the set of three recorded at each time point. Vertical pupil size was measured in millimeters using a standard 30 cm/12-inch ruler held up to the horse’s eye in line with the pupil. Conjunctival hyperemia was graded by the same single observer twice daily (at 07.00 h and 19.00 h) for each eye of each horse during each 10-day study period using a previously published semiquantitative scale [14]. Briefly, the subjective assessment of conjunctival hyperemia was made using a scale from 0 to 3, where 0 = none, 1 = mild, 2 = moderate, and 3 = severe.

[d]Icare Finland Oy, Vaanta, Finland
[e]Manna Pro Products LLC., Chesterfield, Missouri, USA.

Although horses are commonly administered systemic α2-adrenergic receptor agonists such as xylazine and detomidine for sedative purposes and these medications are typically well tolerated, horses participating in this study were closely monitored for signs of adverse reactions to topical ophthalmic brimonidine and a fixed combination of brimonidine and timolol.

Data analysis

Mixed effects linear regression was used to evaluate the main effects of and interactions between treatment (control, brimonidine, or brimonidine-timolol), eye (right or left), time (07.00 h, 13.00 h and 19.00 h), and when appropriate, age, breed, and sex of the horse for IOP and pupil size. Treatment order (brimonidine in phase I and brimonidine-timolol in phase II, or vice versa) was controlled but not analysed. The individual horse was treated as a random effect; all other variables were considered as fixed effects. For IOP measurements, data were analysed in three ways: (1) as the average of all three readings recorded at each time point (i.e. including those with no bar, low bar and medium bar), (2) as individual replicates, or (3) as individual replicates with the medium bar readings excluded. These three methods of data analysis were performed to investigate the robustness of any observed effect across analysis method, to help determine if one method was more sensitive than the others. Analyses were performed using Stata/IC 12.1[f]. All measurements were expressed as mean ± s.d. For all analyses, values of $P \leq 0.05$ were considered significant.

Results

All horses tolerated topical administration of briminodine, brimonidine-timolol or BSS with no evidence of ocular discomfort or conjunctival hyperemia at any time during the study. Using mixed effects linear regression modeling, treatment, eye, time of day, sex, and age effects on IOP were determined at baseline and during the entire measurement period.

Treatment effect on IOP

There was no difference in statistical outcomes between groups when data were analysed three different ways (i.e. as the average of the three recorded measurements, as individual replicates, or as individual replicates with medium bar readings excluded). Thus, only the IOP averaged data will be shown and discussed. Although IOP estimated from measurements taken during baseline, BSS control, brimonidine treatment, brimonidine-timolol treatment, and recovery conditions were all within approximately 1 mm Hg, there was a statistical difference ($P = 0.001$) between predicted mean (95% CI) IOP following treatment with brimonidine of 25.6 (24.2–27.0) mm Hg, in comparison to baseline values 24.7 (23.4–26.1) mm Hg, treatment with brimonidine-timolol 24.6 (23.2–26.0) mm Hg, control measurements with BSS 24.5 (23.2–25.9) mm Hg, and recovery values 24.5 (23.1–25.8) mm Hg (Fig 1).

[f]StataCorp LP, College Station, Texas, USA.

Eye, time of day, sex, and age effects on IOP

At baseline, the left eye had a significantly higher (P<0.001) IOP than the right eye with a predicted mean IOP (95% CI) of 25.4 (24.1–26.7) mm Hg in the left eye and 24.0 (22.7–25.3) mm Hg in the right eye, respectively. During the treatment period, a similar significant (P<0.001) IOP difference between eyes was observed with IOPs of 25.6 (24.5–26.7) and 23.8 (22.7–24.9) mm Hg in the left and right eyes, respectively. At baseline, IOPs did not significantly differ between mares and geldings with estimated IOPs of 25.6 (23.6–27.5) and 24.2 (22.7–25.8) mm Hg, respectively (P = 0.254). During the entire measurement period, mares had significantly greater IOPs than geldings with estimated IOPs of 26.0 (24.4–27.7) and 24.0 (22.6–25.2) mm Hg, respectively (P = 0.038). At baseline, IOP was significantly greater at 13.00 h versus 07.00 h and 19.00 h at 25.8 (24.5–27.1), 24.1(22.8–25.5), and 24.2 (22.8–25.5) mm Hg, respectively (P<0.05, Fig 1). Age did not significantly alter IOP at baseline (P = 0.964) or within the entire measurement period (P = 0.883).

Pupil size

Using mixed effects linear regression modeling, overall treatment effects on vertical pupil size were determined. Treatment with brimonidine, brimonidine-timolol, or BSS did not have a significant effect (P>0.05) on pupil size in comparison to baseline (Fig 2). However, predicted mean vertical pupil size was significantly greater (P<0.001) during the recovery period in comparison to baseline, and treatment with brimonidine, brimonidine-timolol, or BSS-(95% CI) at 6.6 (6.0–7.1), 5.7 (5.2–6.2), 5.7 (5.2–6.3), 5.8 (5.3–6.4), and 5.9 (5.4–6.5) mm, respectively (Fig 2). Predicted mean vertical pupil size was significantly greater (P<0.001) at 07.00 h versus 19.00 h at 7.3 (6.7–7.8) and 4.6 (4.1–5.2) mm, respectively.

Discussion

This study was designed to evaluate brimonidine and brimonidine-timolol as a treatment for equine glaucoma. Specifically, the objective was to evaluate the IOP-lowering efficacy and safety of brimonidine, alone and in conjunction with timolol, as a potential treatment for equine glaucoma by comparing IOP in normal equine eyes treated with brimonidine and brimonidine-timolol to control eyes. We found that brimonidine statistically significantly increased IOP in comparison to baseline, treatment with BSS and brimonidine-timolol, although these differences were of no clinical importance. In contrast, briminodine-timolol did not significantly change IOP in comparison to baseline or BSS. No ocular irritation was identified in any of the horses and brimonidine or brimonidine-timolol did not significantly affect pupil size.

Brimonidine did not seem to cause any negative side effects in normal horses as no horse displayed any conjunctival hyperemia or other noticeable ocular problems during the 5-day treatment period. This observation suggests that it may be safe to use brimonidine in horses with glaucoma, however brimonidine was only administered for five days so the possible long-term side effects were not evaluated. Studies in humans have reported that brimonidine can cause stinging [13], but it is difficult to determine whether the feeling of stinging is a factor with horses. However, no horses showed any blepharospasm or other signs of ocular pain during the treatment period.

Of the two horses that became refractory to treatment and thus were removed from the study after the first study period, one was receiving brimonidine alone and the other was receiving brimonidine-timolol, which is reported to have fewer side effects in people than brimonidine alone [9]. It was more likely that either the intense handling of the horses with only a short break between IOP measurements or the administration of topical ophthalmic medication twice daily was responsible for these two horses becoming refractory to IOP measurement and treatment. The medications were administered using a syringe capped with a needle broken off at the hub to gently spray ophthalmic solutions into the horses' eyes. This method of administration may have been irritating to the horses and resulted in the horses becoming refractive to treatment, although any effect on IOP of refractory behavior should have affected all treatment conditions equally. It is actually impressive that 14 of 16 horses were able to complete both treatment phases of the study without sedation, topical corneal anaesthetic or local nerve block to facilitate manually open the eyelids, and perhaps a testament to the importance of acclimation to the study environment in general and IOP measurement in particular in undertaking studies of IOP in horses.

Although there was a statistically significant increase in IOP associated with brimonidine treatment, it was less than 1 mm Hg. Detecting such a small magnitude of effect is likely associated with the animals generally being well-acclimated to the procedure and the very large number of measurements, as greater than 1800 IOP measurements were collected throughout the study. Furthermore, a 1 mm Hg difference does not represent a clinically significant increase in IOP. It is possible this was a real effect and brimonidine was ineffective at lowering IOP in normal horses. Timolol is generally believed to cause modest reductions in IOP in normal horses but it was ineffective in combination with brimonidine, possibly due to a counter effect of brimonidine or that this set of horses were non-responsive to either drug. In a study of glaucomatous beagles, brimonidine showed a trend in, but was not statistically significant at, lowering IOP [16]. This suggests that some dogs responded to brimonidine with a reduction in IOP but most did not.

An important concept to recognise is that not every person or animal responds with a reduction in IOP even when the drug is generally regarded as highly effective at causing a mean reduction in IOP in the general population [17, 18, 19, 20]. For example, up to 15% of humans do not respond to some of the most effective topical prostaglandin analogues [17, 18, 19, 20]. Studies in normal cats with prostaglandin analogues that are highly effective in humans suggest that the non-responder rate may approach 100% [21]. Similarly, although timolol has been reported to reduce IOP in dogs by 16.1% [22], in a more recent study of the effects of latanoprost, timolol and a latanoprost-timolol combination on pupil size, IOP and heart rate in nine normal dogs, there was no decrease in IOP in 11% of the dogs when treated with latanoprost alone, and there was no decrease in IOP in any of the nine dogs when treated with timolol alone [23]. Therefore, it is possible that the individuals in this population of horses were not responsive to timolol whereas in the previous study enough horses were responsive so as to lower mean IOP a statistically significant amount.

Eye, time of day, sex, and age were evaluated as potentially having effects on IOP. The small but statistically greater IOP in the left versus right eye may be associated with laterality of handling, as most horses are used to being handled on their left, or near, side. There is some

evidence that horses prefer to use their left eye for assessment and evaluation [24], and therefore perhaps horses in this study exhibited more of a squinting artifact that affected IOP measurement in the left eye more than the right. IOP was greatest midday, consistent with previously reported circadian IOP rhythmicity in horses showing the highest IOP during peak light [25]. There was no age effect during the entire measurement period, and no sex effect on IOP at baseline, however mares exhibited a 2 mm Hg greater IOP than geldings during the treatment phase. The reason for this is unknown.

Horses included in the study were fed at different times each morning. Feeding sometimes interrupted IOP measurement and treatment administration. In addition, because the horses were in open pens, they were able to observe movement of other horses around the farm. Excitement associated with feeding and with movement of other horses could have affected measured IOP, however, the statistically significant and consistent diurnal change in IOP indicated that the data were reflective of what occurs physiologically with IOP, and also suggest that the measurements were not altered sporadically by fluctuations in the amounts of stress experienced by the horses.

There were no significant effects of brimonidine or brimonidine-timolol on vertical pupil size in this study. However, as expected, vertical pupil size varied throughout the day, being the largest in the morning and significantly decreased in the afternoon and early evening. This is most likely associated with changes in ambient lighting caused by differential shading based on the height of the sun (i.e. the pens housing the horses were the most shaded when the sun was directly overhead in the middle of the day).

Even though treatments containing brimonidine did not lower IOP in normal horses, it is possible that horses with glaucoma will show an IOP-lowering effect. While brimonidine has been shown to be effective at decreasing IOP in glaucomatous human patients [10, 11], it was ineffective at significantly decreasing IOP in glaucomatous beagles [16]. Changes in IOP in normal animals can be subtle and the results are usually magnified in glaucomatous animals [26–28]. Thus, it would be ideal to conduct a clinical trial in glaucomatous horses to see if brimonidine or brimonidine-timolol decreased IOP in equine patients.

In conclusion, this study showed brimonidine and brimonidine-timolol were safe to use in normal horses, with no adverse systemic or ocular effects noted. However, its efficacy as a treatment for horses with glaucoma is questionable as brimonidine statistically significantly increased IOP in normal horses, although this was less than 1 mm Hg and considered clinically insignificant. It is unknown whether horses with glaucoma would achieve some IOP-lowering benefit from use of brimonidine with or without timolol; further studies using clinical patients are warranted.

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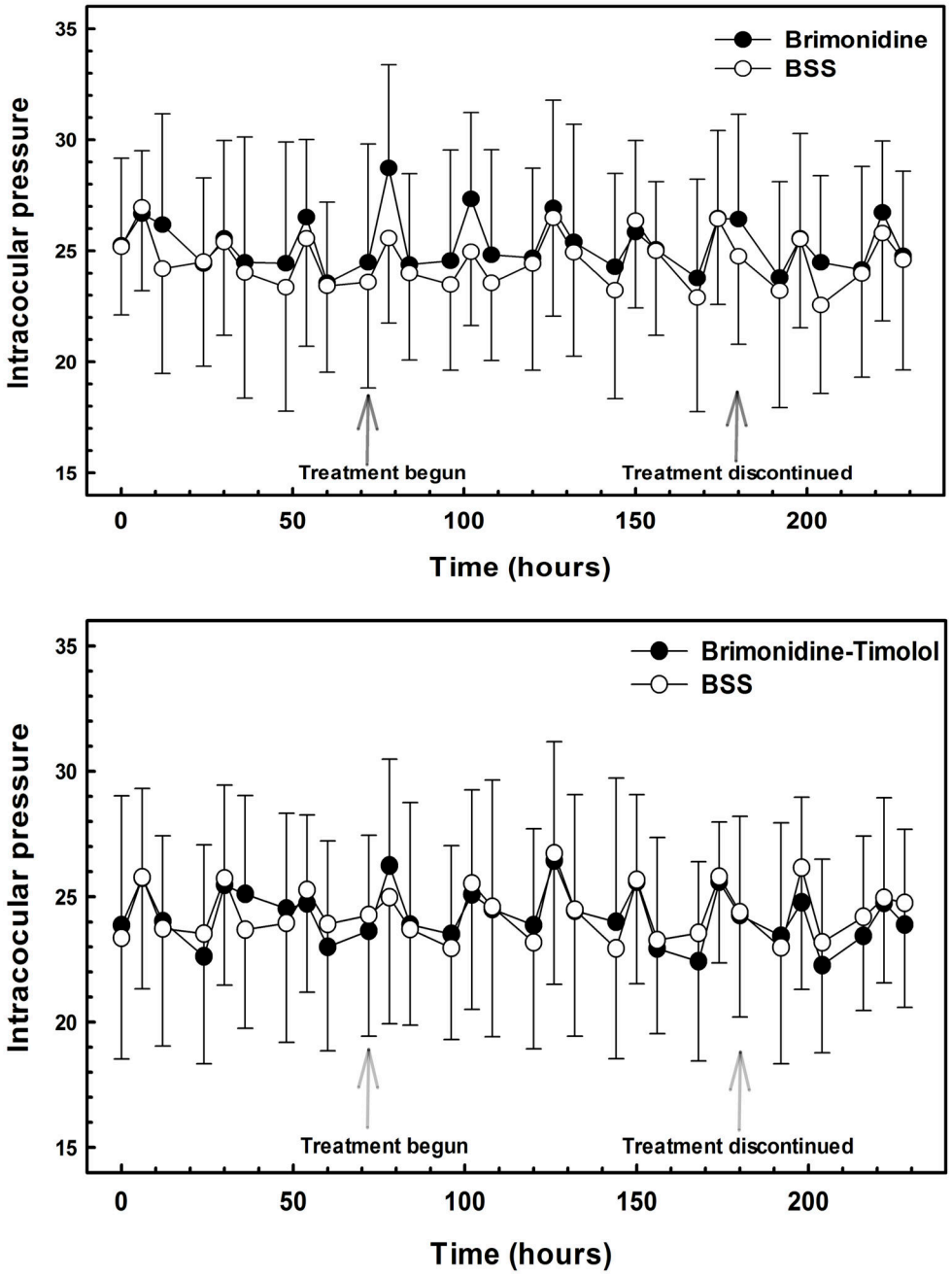

Fig 1.

Mean ± s.d. IOPs measured three times daily (07.00 h, 13.00 h and 19.00 h) prior to, during and following administration at 07.00 h and 19.00 h of topical ophthalmic 0.2% brimonidine (**A**) or 0.2% brimonidine tartrate/0.5% timolol maleate (**B**) in one eye and BSS in the opposite eye every 12 h to 15 horses. A balanced crossover design was used in which 8 horses received brimonidine or brimonidine-timolol. After an 18 day washout period, the alternative treatment was administered. Predicted mean IOP following treatment with brimonidine was significantly greater ($P = 0.001$) than baseline values, control measurements with BSS and recovery values. However, there was no significant difference

($P>0.05$) between brimonidine-timolol, baseline values, control measurements with BSS and recovery values. Intraocular pressure was significantly greater ($P<0.05$) at 13.00 h versus 07.00 h and 19.00 h.

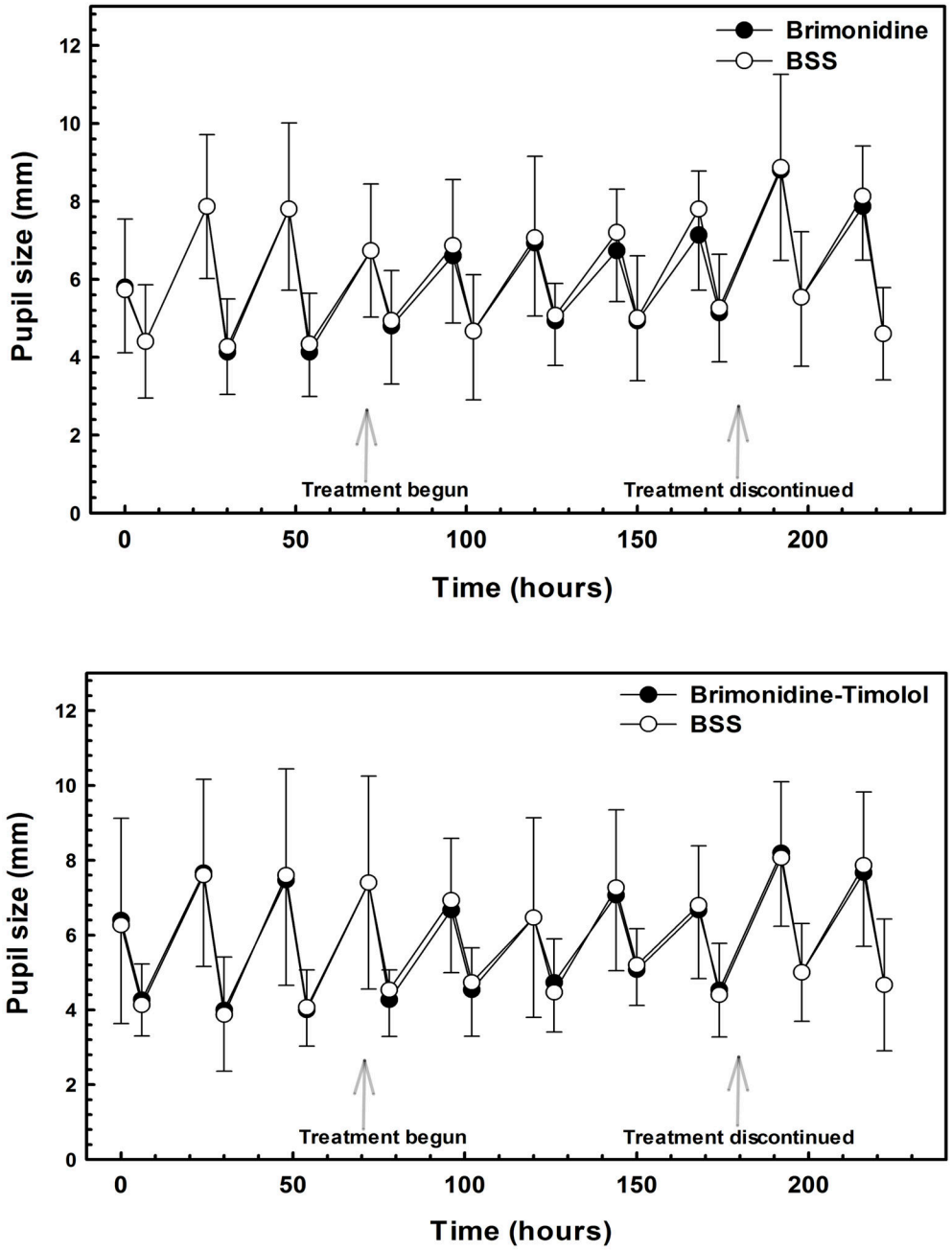

Fig 2.

Mean ± s.d. pupil sizes measured twice daily (07.00 h and 19.00 h) prior to, during and following administration of topical ophthalmic 0.2% brimonidine (**A**) or 0.2% brimonidine tartrate/0.5% timolol maleate (**B**) in one eye and BSS in the opposite eye every 12 h to 15 horses. A balanced crossover design was used in which 8 horses received brimonidine or brimonidine-timolol. After an 18 day washout period, the alternative treatment was administered. Brimonidine and brimonidine-timolol did not have a significant effect ($P>0.05$) on pupil size throughout the study period. Pupil size was significantly ($P<0.001$)

decreased during the recovery period for all treatments. Pupil size was significantly ($P<0.001$) greater at 07.00 h versus 19.00 h for all treatments.