EQUINE VETERINARY JOURNAL
Equine vet. J. (2008) **40** (7) 662-665
doi: 10.2746/042516408X297426

Effects of topical administration of 1% brinzolamide on intraocular pressure in clinically normal horses

S. E. GERMANN*, F. L. MATHEIS, A. RAMPAZZO, D. BURGER†, M. ROOS‡ and B. M. SPIESS

Ophthalmology Section, Equine Department Vetsuisse Faculty, University of Zurich, 8057 Zurich; †Swiss National Stud Farm, 1580 Avenches; and ‡Biostatistics Unit, ISPM, University of Zurich, 8006 Zurich, Switzerland.

Keywords: horse; eye; glaucoma; carboanhydrase inhibitor; brinzolamide; intraocular pressure

Summary

Reasons for performing study: **Only few drugs with limited efficacy are available for topical treatment of equine glaucoma.**

Objective: **To evaluate the effect of topical administration of 1% brinzolamide on intraocular pressure (IOP) in clinically normal horses.**

Methods: **Healthy mature horses (n = 20) with normal ocular findings, were studied. The IOP was measured 5 times daily (07.00, 11.00, 15.00, 19.00 and 23.00 h) over 10 days. On Days 1 and 2, baseline values were established. On Days 3–5 one eye of each horse was treated with one drop of 1% brinzolamide every 24 h immediately following the 07.00 h measurement. On Days 6–8 the same eye was treated with 1% brinzolamide every 12 h (07.00 and 19.00 h). Measurements on Days 9 and 10 documented the return of IOP to baseline values. Statistical analysis of the data was performed.**

Results: **In the treated eye a significant decrease in IOP compared to baseline values was noted during both the 24 and 12 h dosing periods (P<0.001). During the once-daily treatment protocol an IOP reduction of 3.1 ± 1.3 mmHg (14%) from baseline was recorded. During the twice-daily protocol a total IOP reduction of 5.0 ± 1.5 mmHg (21%) was achieved.**

Conclusion: **Intraocular pressure was significantly decreased by 1% brinzolamide in a once-daily and a twice-daily treatment protocol in normotensive eyes. These findings suggest that brinzolamide might also be effective in horses with an elevated IOP.**

Potential relevance: **This drug may be useful for treatment of equine glaucoma.**

Introduction

Different types of glaucoma, such as congenital, primary and secondary have been described in the horse but secondary is the most common, typically as a result of trauma or chronic intraocular inflammation associated with equine recurrent uveitis (Wilkie and Gilger 2004).

Treatment is directed towards either improving aqueous humour outflow or reducing aqueous humour production. The main goal in treatment of affected animals is to maintain intraocular pressure (IOP) within a range compatible with intraocular health and sustained visual function (Willis *et al*. 2001a). Medical and surgical treatment options have been described. Medical treatment in equine glaucoma concerns mainly the use of topical agents. Some conflicting data have been published concerning the ability of several topical formulations to decrease IOP in healthy horses. One percent atropine sulphate showed a mean IOP decrease of 11.2% in one study (Herring *et al*. 2000), but no IOP reduction in another (Mughannam *et al*. 1999). The prostaglandin analogue 0.005% latanoprost decreased IOP by 5% in male and 17% in female horses in one study (Willis *et al*. 2001b), but failed to have a lowering effect in a second study (Davidson *et al*. 2002). In both studies, pronounced negative side effects (epiphora, blepharospasm and blepharedema) were associated with the use of the drug. The β-blocker 0.5% timolol maleate decreased IOP by 17% in a single-dose application and by 27% in a twice-daily application during several days (van der Woerdt *et al*. 2000). The carbonic anhydrase inhibitor (CAI), 2% dorzolamide hydrochloride alone or in combination with 0.5% timolol maleate, produced a significant IOP reduction in twice-daily administration. However, the decrease was not substantial (mean IOP decrease compared to baseline <2 mmHg) and dorzolamide or dorzolamide-timolol could be recommended only as adjunctive treatment for horses with glaucoma (Willis *et al*. 2001a). As only few drugs with limited efficacy are available for topical treatment, newer agents are under investigation for their use in equine glaucoma.

Brinzolamide is a sulphonamide-based CAI closely related to dorzolamide, which was formulated for the management of human glaucoma and approved for ophthalmic use in 1998 (Sugrue 2000; Herkel and Pfeiffer 2001; Willis *et al*. 2002). Its mechanism of action corresponds to other CAIs and lies in the suppression of carbonic anhydrase in the ciliary epithelium, which results in a lower production of aqueous humour (Derick 1994). Experimental work with rabbits indicates that brinzolamide readily penetrates the eye by both corneal and scleral routes and reaches high concentrations within the ciliary body. Furthermore, it has a relatively long half-life of several days in iris-ciliary body, choroid, retina and lens, which leads to an extended action in the eye (De Santis 2000). Comparisons between brinzolamide and dorzolamide in man showed that the former had a significantly

*Author to whom correspondence should be addressed.
[Paper received for publication 18.12.07; Accepted 20.01.08]

higher tolerability than the latter drug (Silver and Group 2000; Sugrue 2000); and that less frequent application resulted in an equal IOP decrease (De Santis 2000; Sall and Group 2000). A further advantage is the topical application of the drug, which eliminates the typical side effects associated with systemically administered CAIs (De Santis 2000). Brinzolamide was shown to reduce IOP in dogs (Whelan *et al*. 1999), but did not significantly affect IOP in cats in a short-term setting (Gray *et al*. 2003).

To the authors' knowledge, there are no previous reports on the use of 1% brinzolamide in the horse. The aim of this study was therefore to investigate the IOP lowering properties of this drug in a once- and a twice-daily treatment protocol in healthy horses.

Materials and methods

Pilot study

A pilot study with 9 horses (results not shown) was conducted in order to calculate appropriate study number, measurement intervals and to evaluate the reproducibility of the measurements. Additionally the pilot study provided the opportunity to train the measurement operator (S.E.G.) and to standardise the procedure. For both the pilot as well as the final study, governmental licences for animal experiments were obtained.

Animals

The pilot study revealed that, for a study with 90% power and 5% significance level, 20 horses should be adequate. On this basis, 20 mature horses (16 stallions, 4 mares) were included in the study population. All 16 stallions were Franches-Montagnes horses; among the mares there were 2 Standardbreds, one Franches-Montagnes and one Swiss Warmblood. Age range was 3–15 years (mean ± s.d. = 7.1 ± 3.26 years). The study was conducted on the Swiss National Stud Farm. During the study, the horses were kept in their usual environment and feeding and exercise regime was continued as usual. Prior to initiation of the study all horses underwent a thorough clinical and ophthalmological examination. The anterior segment was evaluated by slit lamp biomicroscopy (Kowa SL-14)[1], the posterior segment by direct ophthalmoscopy using a focal light source (Augenuntersuchungslampe nach Ammann)[2]. During this period the horses were handled several times and familiarised with the examination methods used subsequently. Only healthy horses with normal ocular findings were included in the study population.

Measurement of IOP

Rebound tonometry (Tonovet)[3] was used for the IOP measurements according to specifications of the manufacturer. The measurements were taken in a calm environment, the head was maintained in an upright position by gentle manual restraint and the eyelids manipulated minimally in order to avoid pressure to the globe. No topical anaesthetic was required and the examination was well tolerated by the animals. Neither sedation, nor blocking of the auriculopalpebral nerve was necessary. The measurements were always taken by the same individual (S.E.G.), only readings with s.d. <1.8 mmHg were taken into consideration. During the study period of 10 days IOP was assessed 5 times daily bilaterally in all horses (at 07.00, 11.00, 15.00, 19.00 and 23.00 h).

Study period

For means of comparison of results with existing data, the following study design was chosen to be similar to other authors (Willis *et al*. 2001a). During the first 2 days (*period 0*) of the study period, baseline values were established in order to document individual normal values for each horse and detect eventual diurnal IOP fluctuations in the study population. From Days 3–5 (*period 1*), one eye of each horse was treated with one drop of 1% brinzolamide (Azopt)[4] (approximately 33.5 μl = 335 μg brinzolamide) at 07.00 h immediately after the IOP measurement. The same eye was always treated, the contralateral eye remained untreated. From Days 6–8 (*period 2*) the horses were treated twice-daily at 07.00 and 19.00 h with one drop of 1% brinzolamide always in the same eye. On Days 9 and 10 (follow-up period; *period 3*) drug administration was stopped, but IOP monitoring continued in order to assess return of IOP to baseline values.

Statistical analysis

To investigate the effects of 1% brinzolamide in the studied horse population, mean IOP values were calculated for each of the 5 time points for either the treated or the untreated eye in each horse. A global comparison using repeated measures analysis ANOVA (together with Greenhouse-Geisser correction) was made for the whole study period. Eventual diurnal IOP fluctuations and the confounding effect of age were also evaluated with ANOVA. Data of IOP were expressed as mean ± s.d. for the different study *periods 0, 1, 2* and *3*. Paired *t* test was used to compare the IOP difference between baseline and the following study periods in either treated or untreated eyes. Moreover, the IOP difference between treated and untreated eye in each horse was analysed by a paired *t* test. Significance was set at $P \leq 0.05$. Data analysis was performed with Stat View, version 5.0.1 and SPSS, version 11 for Mac OS.

Results

Horses and treatment tolerance

All horses tolerated the treatment with 1% brinzolamide well and showed no clinical signs of ocular discomfort or systemic side effects.

Changes in IOP

Repeated measures analysis ANOVA showed that there were no statistically significant diurnal IOP fluctuations during the baseline period. IOP values for the baseline period (*0*) among the 20 horses ranged 16–36 mmHg, mean 22.8 ± 2.3 mmHg. In the treated eye IOP was 19.7 ± 2.1 mmHg, 17.8 ± 2.0 mmHg and 20.9 ± 2.0 mmHg for *periods 1* to *3*, respectively. Corresponding values for the untreated eye were 21.0 ± 1.9 mmHg, 19.2 ± 1.8 mmHg and 21.2 ± 2.0 mmHg. Mean IOP values for the treated eye, over the 4 study periods, are shown graphically in Figure 1.

Repeated measures analysis ANOVA revealed that the IOP decrease in the treated eye was statistically significant in both the once- (*period 1*) and twice-daily dosing periods (*2*) ($P<0.001$). The IOP decreased from *period 0* to *period 1* was 3.1 ± 1.3 mmHg (14%), and from *period 0* to *period 2,* 5.0 ± 1.5 mmHg (21%). In

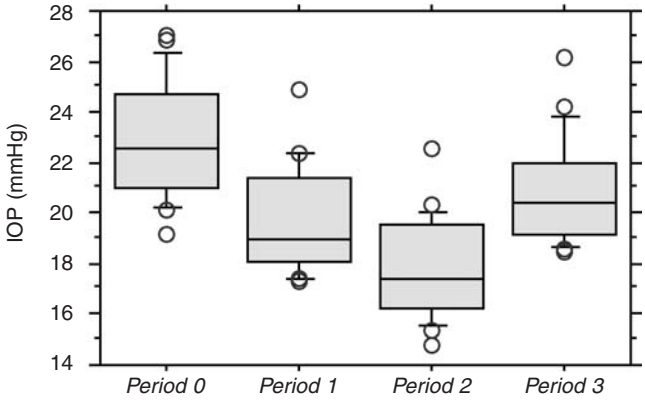

Fig 1: Box plot presentation of mean intraocular pressure (IOP) values with s.d. (mmHg) for the treated eye over the different study periods (0 = baseline, 1 = brinzolamide q. 24 h, 2 = brinzolamide q. 12 h and 3 = follow-up).

the untreated eye an IOP decrease was also observed, less than in the treated eye, but still significant (P<0.001). In this eye the IOP reduction from *period 0* to *period 1* was 1.8 ± 1.1 mmHg, from *period 0* to *period 2*, 3.5 ± 1.2 mmHg. Once treatment was discontinued, IOP started to increase again and at the end of *period 3* the horses had nearly reached their initial baseline values. It could be shown with paired *t* test that the last measurement of *period 3* was not significantly different from the baseline value in either eye. Paired *t* test also revealed that the difference in IOP reduction between the treated and the untreated eye in the same animal was statistically significant, with the IOP in the treated eye being significantly lower than in the untreated eye (P<0.0001).

Repeated measures analysis ANOVA showed that the age of the horses did not have a statistically significant influence on IOP and IOP decrease under treatment with 1% brinzolamide.

Discussion

The increasing diagnosis of glaucoma in horses may be due to higher awareness of veterinarians of the characteristic appearance of the disease and access to portable tonometers (Willis *et al*. 2001a). The Tonovet rebound tonometer used for IOP measurements in this study was reliable, practical and easy to use. Its use was well tolerated by the horses, with no reaction normally seen to the probe touching the cornea during measurement.

Mean ± s.d. baseline IOP values 22.8 ± 2.3 are comparable to results of Knollinger *et al*. (2005) who assessed normal IOP in horses by use of the Tonovet tonometer (22.1 ± 5.9 mmHg). The spread of the baseline values in the study population was relatively broad; however, when evaluated horse by horse different measurements showed only little variation. It was therefore chosen to evaluate data of IOP measurements with patient specific paired tests. In accordance to previous investigations in horses no diurnal IOP fluctuations could be detected (van der Woerdt *et al*. 1998; Mughannam *et al*. 1999; Willis *et al*. 2001a; Davidson *et al*. 2002).

The age of the horses did not significantly affect IOP during treatment with 1% brinzolamide. It was supposed that sex would not have a statistically significant influence either, but statistical analysis could not be performed due to the small number of mares in the study.

In the studied horse population, 1% brinzolamide reduced the IOP significantly in a once- and a twice-daily treatment protocol. The large IOP decrease in *period 2* might be due to the higher dosing frequency of brinzolamide. However, it must be considered that there was no washout time between *periods 1* and *2* and that the further IOP decrease could also be caused partially by some cumulative or persisting effect of the drug. Perhaps, if either treatment would have been prolonged, an even more pronounced IOP reduction would have been reached. IOP development under more long-term treatment conditions, as well as the effects of higher frequency of drug application or greater amount of drug per application, still need to be determined in horses.

As in several previous veterinary and human studies evaluating glaucoma drugs, an IOP decrease was observed in both treated and untreated eyes (Opremcak and Weber 1985; Gum *et al*. 1991; King *et al*. 1991; Wilkie and Latimer 1991a,b; Willis *et al*. 2001a). This IOP decrease in the contralateral eye is explained as a result of systemic absorption of the drug (King *et al*. 1991; Willis *et al*. 2001a). Studies investigating pharmacokinetics of brinzolamide and the related CAI dorzolamide in animals and man, detected drug levels in red blood cells, plasma and urine and confirmed some degree of systemic absorption (Wilkerson *et al*. 1993; Strahlman *et al*. 1996; Maren *et al*. 1997; Hall *et al*. 1999; De Santis 2000). In the present clinical study chemistry profiles for analysis of blood levels or urinary excretion that could confirm a systemic distribution of the drug were not performed.

The IOP reduction obtained with once- and twice-daily administration of 1% brinzolamide in this study is superior to data published concerning the use of the related CAI dorzolamide in the horse, where a slight IOP decrease was only achieved in the twice-daily dosing regime (Willis *et al*. 2001a). Based on comparison between the 2 agents in man, some major advantages were attributed to the more recent CAI brinzolamide. It could be shown that due to its formulation at physiological pH 7.5, 1% brinzolamide was significantly better tolerated than 2% dorzolamide, which has a much lower pH of 5.6 (Silver and Group 2000; Sugrue 2000). The pH value of the human precorneal tear film is 7.6, in the horse this is pH 8.33 ± 0.15 (8.0–8.6) (Fischer and Wiederholt 1982; Lowe and Crispin 2003). It may also be assumed that in horses a more physiological pH formulation would increase tolerance. Treatment comfort is an important factor affecting patient cooperation and owner compliance. Another favourable point demonstrated in man was that 1% brinzolamide instilled twice-daily results in the same IOP decrease as 1% brinzolamide instilled 3-times-daily and 2% dorzolamide instilled 3-times-daily (De Santis 2000; Sall and Group 2000). Less frequent drug application again leads to higher owner compliance and better treatment results. The effects of 3-times-daily application of 1% brinzolamide to horses were not investigated in this study, but the circumstances may be comparable to man.

In the present study, a statistically significant IOP decrease was achieved with once- and twice-daily application of 1% brinzolamide in a population of healthy equine patients. The results obtained in normal equine eyes may allow conclusions to be drawn concerning the glaucomatous equine eye. According to studies evaluating topical CAIs in man and dogs, the IOP reduction tends to be even more substantial in glaucomatous than in healthy eyes (King *et al*. 1991; Lippa *et al*. 1992; Strahlmann *et al*. 1992; Wilkerson *et al*. 1993). However, further controlled

clinical studies are necessary to determine the effect of brinzolamide in equine glaucoma cases.

In this study, it was demonstrated, that once- and twice-daily 1% brinzolamide was effective for reducing IOP in a study population of healthy horses and that the drug was well tolerated. These findings suggest that 1% brinzolamide might be useful in equine glaucoma, either alone or as an adjunct to another IOP lowering modality.

Acknowledgements

The authors would like to express their thanks to Alcon Pharmaceuticals Ltd., Hunenberg, Switzerland for providing free samples of 1% brinzolamide solution (Azopt), as well as to the Provet vet.-med. Center, Lyssach, Switzerland, for providing a spare Tonovet tonometer and measurement probes for use in the study. Neither of the authors has any commercial or proprietary interest in the products mentioned.

Manufacturers' addresses

[1]Kowa Company Ltd., Tokyo, Japan.
[2]Heine, Herrsching, Germany.
[3]Tiolat Ltd., Helsinki, Finland.
[4]Alcon Pharmaceuticals Ltd., Hunenberg, Switzerland.

References

Davidson, H.J., Pinard, C.L., Keil, S.M., Brightman, A.H. and Sargeant, J.M. (2002) Effect of topical ophthalmic latanoprost on intraocular pressure in normal horses. *Vet. Ther.* **3**, 72-80.

Derick, R.J. (1994) Glaucoma therapy, section one: Carbonic anhydrase inhibitors. In: *Havener's Ocular Pharmacology*, 6th edn., Eds: T.F. Mauger and E.J. Craig, Mosby, St Louis. pp 172-180.

De Santis, L. (2000) Preclinical overview of brinzolamide. *Survey Ophthalmol*. **44**, 119-129.

Fischer, F.H. and Wiederholt, M. (1982) Human precorneal tear film pH measured by microelectrodes. *Graefes Arch. Clin. Expt. Ophthalmol*. **218**, 168-170.

Gray, H.E., Willis, A.M. and Morgan, R.V. (2003) Effects of topical administration of 1% brinzolamide on normal cat eyes. *Vet. Ophthalmol*. **6**, 285-290.

Gum, G.G., Larocca, R.D. and Gelatt, K.N. (1991) The effect of topical timolol maleate on intraocular pressure in normal beagles and beagles with inherited glaucoma. *Prog. vet. Comp. Ophthalmol*. **1**, 141-149.

Hall, R., Havner, G. and Baker, J. (1999) Brinzolamide. In: *Analytical Profiles of Drug Substances and Excipients*, Ed: H. Brittain, Academic Press, San Diego. pp 47-96.

Herkel, U. and Pfeiffer, N. (2001) Update on topical carbonic anhydrase inhibitors. *Curr. Opin. Ophthalmol*. **12**, 88-93.

Herring, I.P., Pickett, J.P., Champagne, E.S., Troy, G.C. and Marini, M. (2000) Effect of topical 1% atropine sulfate on intraocular pressure in normal horses. *Vet Ophthalmol*. **3**, 139-143.

King, T.C., Gum, G.G. and Gelatt, K.N. (1991) Evaluation of a topically administered carbonic anhydrase inhibitor (MK-927) in normotensive and glaucomatous beagles. *Am. J. vet. Res*. **52**, 2067-2070.

Knollinger, A.M., La Croix, N.C., Barrett, P.M. and Miller, P.E. (2005) Evaluation of a rebound tonometer for measuring intraocular pressure in dogs and horses. *J. Am. vet. med. Ass*. **227**, 244-248.

Lippa, E.A., Carlson, L.E. and Ehinger, B. (1992) Dose response and duration of action of dorzolamide a topical carbonic anhydrase inhibitor. *Arch. Ophthalmol*. **110**, 495-499.

Lowe, R.C. and Crispin, S.M. (2003) Normal equine tear pH as measured with pH paper. In: *Programs and Abstracts of the Joint Meeting of BrAVO, ECVO, ESVO, ISVO*, Cambridge.

Maren, T., Conroy, C., Wynns, G. and Levy, N. (1997) Ocular absorption, blood levels, and excretion of dorzoamide, a topically active carbonic anhydrase inhibitor. *J. Ocul. Pharmacol. Ther.* **12**, 23-30.

Mughannam, A.J., Buyukmihci, N.C. and Kass, P.H. (1999) Effect of topical atropine on intraocular pressure and pupil diameter in the normal horse eye. *Vet. Ophthalmol*. **2**, 213-215.

Opremcak, E.M. and Weber, P.A. (1985) Interaction of timolol and caffeine on intraocular pressure. *J. Ocul. Pharmacol. Ther.* **1**, 227-234.

Sall, K. and Group, B.P.T.S. (2000) The efficacy and safety of brinzolamide 1% ophthalmic suspension (Azopt) as a primary therapy in patients with open angle glaucoma or ocular hypertension. *Survey Ophthalmol*. **44**, 155-162.

Silver, L.H. and Group, B.C.S. (2000) Ocular comfort of brinzolamide 1% ophthalmic suspension compared with dorzolamide 2% ophthalmic solution: results from two multicenter comfort studies. *Survey Ophthalmol*. **44**, 141-145.

Strahlmann, E.R., Tipping, R. and Vogel, R. (1992) A double-masked, randomized 1-year study comparing dorzolamide (Trusopt), timolol and betaxolol. *Arch. Ophthalmol*. **113**, 1009-1016.

Strahlman, E., Tipping, R. and Vogel, R. (1996) A six-week dose-response study of the ocular hypotensive effect of dorzolamide with a one-year extension. Dorzolamide Dose-Response Study Group. [erratum appears in *Am. J. Ophthalmol*. (1996) **122**, 928]. *Am. J. Ophthalmol*. **122**, 183-194.

Sugrue, M.F. (2000) Pharmacological and ocular hypotensive properties of topical carboanhydrase inhibitors. *Progr. Retina Eye Res*. **19**, 87-112.

van der Woerdt, A., Gilger, B.C., Wilkie, D.A., Strauch, S.M. and Orczeck, S.M. (1998) Normal variation in, and effect of 2% pilocarpine on, intraocular pressure and pupil size in female horses. *Am. J. vet. Res*. **59**, 1459-1462.

van der Woerdt, A., Wilkie, D.A., Gilger, B.C., Strauch, S.M. and Orczeck, S.M. (2000) Effect of single- and multiple-dose 0.5% timolol maleate on intraocular pressure and pupil size in female horses. *Vet. Ophthalmol*. **3**, 165-168.

Whelan, N.C., Welch, P., Pace, A. and Brienza, C.A. (1999) A comparison of the efficacy of topical brinzolamide and dorzolamide alone and in combination with oral methazolamide in decreasing normal canine intraocular pressure. In: *30th Annual Meeting of the American College of Veterinary Ophthalmologists*. pp 267-268.

Wilkerson, M., Cyrlin, M., Lippa, E.A., Esposito, D., Deasy, D., Panebianco, D., Fazio, R., Yablonski, M. and Shields, M.B. (1993) Four-week safety and efficacy study of dorzolamide, a novel, active topical carbonic anhydrase inhibitor. *Arch. Ophthalmol*. **111**, 1343-1350.

Wilkie, D.A. and Gilger, B.C. (2004) Equine glaucoma. In: *Updates in Ophthalmology*, Ed: T.J. Cutler, Elsevier Saunders, Philadelphia. pp 381-391.

Wilkie, D.A. and Latimer, C.A. (1991a) Effects of topical administration of timolol maleate on intraocular pressure and pupil size in dogs. *Am. J. vet. Res*. **52**, 432-435.

Wilkie, D.A. and Latimer, C.A. (1991b) Effects of topical administration of timolol maleate on intraocular pressure and pupil size in cats. *Am. J. vet. Res*. **52**, 436-440.

Willis, A.M., Diehl, K.A. and Robbin, T.E. (2002) Advances in topical glaucoma therapy. *Vet. Ophthalmol*. **5**, 9-17.

Willis, A.M., Robbin, T.E., Hoshaw-Woodard, S., Wilkie, D.A. and Schmall, M.L. (2001a) Effect of topical administration of 2% dorzolamide hydrochloride or 2% dorzolamide hydrochloride-0.5% timolol maleate on intraocular pressure in clinically normal horses. *Am. J. vet. Res*. **62**, 709-713.

Willis, A.M., Diehl, K.A., Hoshaw-Woodard, S., Kobayashi, I., Vitucci, M.P. and Schmall, L.M. (2001b) Effects of topical administration of 0.005% latanoprost solution on eyes of clinically normal horses. *Am. J. vet. Res*. **62**, 1945-1951.

Author contributions This study was initiated, conceived, planned and written by S.E.G., A.R., D.B., M.R. and B.M.S. It was executed by S.E.G., F.L.M. and D.B. and statistics by S.E.G. and M.R.