EFFECTS OF THE COMBINED ADMINISTRATION OF BRINZOLAMIDE 1% AND TIMOLOL 0.5% EYE DROPS ON THE CIRCADIAN RHYTHM OF INTRAOCULAR PRESSURE AND PUPIL DIAMETER AND HEART RATE IN HEALTHY CATS

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Abstract

The aim of this study was to investigate the effects of brinzolamide 1% eye drops administered three times daily (q8h) in combination with timolol 0.5% eye drops administered two times daily (q12h) on the circadian rhythm of intraocular pressure (IOP) and on heart rate and pupil diameter in healthy cats.

In this prospective, randomized, blind study, IOP, horizontal pupil diameter and heart rate were assessed in ten healthy cats. Adjustment period values, placebo values and values during four different treatments (among which brinzolamide and brinzolamide + timolol) were obtained. During the placebo period the cats received an artificial tear drop in one randomly assigned eye and during the treatment periods they received medication in the same eye and an artificial tear drop in the untreated eye. Measurements were performed every three hours for five days every treatment period and the washout period between treatments lasted three days. Values were analysed using linear mixed-effect models.

The overall mean IOP in the placebo period was 14.2 ± 0.21 mmHg and mean IOP following topical administration of brinzolamide and timolol was 11.3 ± 0.17 mmHg. The mean reduction in IOP of 20% as compared to the placebo period was statistically significant ($P < 0.0001$). However, the additional 5% reduction in IOP as compared to treatment with brinzolamide alone (11.9 ± 0.15 mmHg) was not statistically significant ($P = 0.30$). Compared to the placebo period pupil diameters were significantly reduced from 9 AM until 12 PM, with maximal reduction of 1.25 ± 0.24 mm (19%) at 9 PM. Treatment with brinzolamide and timolol did not significantly alter mean overall heart rates, 152 ± 1.3 bpm during both the placebo period and during brinzolamide and timolol treatment period. No ocular irritation was observed after artificial teardrop, brinzolamide eye drop or timolol eye drop administration.

Although the mean reduction in IOP of 20% after q8h brinzolamide 1.0% eye drops and q12h timolol 0.5% eye drops in healthy cats was significant, further investigation in glaucomatous cats is necessary to define clinical significance in glaucomatous cats. Besides, the addition of timolol to the treatment did not significantly decrease IOP more than brinzolamide alone.

Introduction

Glaucoma is a disease that is characterised by the degeneration of the optic nerve and retina, mostly associated with an elevated intraocular pressure (IOP) and can lead to ocular discomfort and blindness (McLellan 2011). Glaucoma is more often diagnosed in older cats. A study estimated 1% of cats older than seven years may become glaucomatous and another study found that 1 in 367 of all cats presenting to University Teaching Hospitals was diagnosed as glaucomatous. (McLellan 2011, Kiland 2016)

Clinical signs of an increased IOP are mostly not noticed until in a late stage of glaucoma. In one retrospective study 73% of glaucomatous cats was already blind at the time of presentation. Clinical signs that may be observed are loss of vision, pupillary dilatation, mild corneal edema and conjunctival hyperemia. (Willis, Diehl et al. 2002, Kiland 2016, McLellan 2011) IOP can differ during days and between days. A study described a difference in the order of 4 mmHg during a day in healthy cats, due to circadian fluctuation. Peak values were found between 9 PM and 12 AM and low values were found between 9 AM and 6 PM (Sole 2007) Therefore measurements should be done both during the day and at night to get a representative overview of the IOP and repeated IOP measurements in patients should be done at the same time points.

Feline glaucomas are divided in primary and secondary glaucomas. Only 2-5% of the feline glaucoma cases is diagnosed as primary glaucoma, however, it may be underdiagnosed. Primary glaucoma may be heritable and there is a strong breed predisposition. The other 95-98% of the feline glaucoma cases is diagnosed as secondary glaucoma. In secondary glaucomas the elevated IOP is often a result of impaired aqueous humor outflow, caused by other disease processes that alter aqueous humor dynamics. Those disease processes include uveitis, trauma, neoplasia and intra-ocular haemorrhage. (McLellan 2011)

Systemic treatment of glaucoma can cause side effects, hence topical treatment is preferred (Gray 2003). There are barely medications registered for glaucomatous cats, hence human topical glaucoma medications are often prescribed to cats, for example, brinzolamide 1% and timolol 0,5% eye drops.

Brinzolamide is a carbonic anhydrase inhibitor (CAI). Topically administered CAIs inhibit the enzyme carbonic anhydrase in the ciliary processes of the uvea. The formation of bicarbonate decreases, subsequently reducing passive sodium and fluid transport. As a result of the decreased aqueous humor production IOP decreases. Only one study and one abstract on the effects of brinzolamide in cats has been published. In this study brinzolamide was administered topically to normotensive cat eyes twice daily (q12h), but it did not significantly reduce IOP. There were no clinically relevant side effects found either. (Gray 2003) In the abstract brinzolamide was administered three times daily (q8h). A significant reduction in IOP of 3.1 ± 0.38 mmHg was found in glaucomatous cats. In healthy cats brinzolamide did not significantly reduce IOP. Unfortunately the full article has not been published. (McLellan, Lin et al. 2009) Other studies investigated the effects of brinzolamide on normotensive dogs, horses and humans and found significant IOP reductions. (Willis, Diehl et al. 2002) The little side effects are also mentioned in those studies.

Another topical glaucoma medication is timolol, a nonselective beta-blocker that reduces aqueous humor production by inhibiting synthesis of cyclic AMP in the ciliary body (Kiland 2016). In addition, timolol may inhibit the beta-adrenergic nerve fibers that inhibit the iris sphincter muscle, resulting in miosis of the treated eye (Kiland 2016). Furthermore, beta-blockers may act as neuroprotectants. Beta-blockers reduce influx of sodium and calcium by blocking the channels. The reduced influx of sodium protects the ganglion cell axon and the reduced influx of calcium protects the ganglion cell body. However, methods of drug delivery to the optic nerve head have not been investigated yet and are necessary to realise the neuroprotective effect. A systemic effect of timolol when applied topically may cause neuroprotection. (Osborne, Wood et al. 2005) Topically administered timolol eye drops may cause side effects after systemic absorption by antagonising beta-adrenergic receptors. Therefore topical administered timolol eye drops can decrease heart rate. (Colasanti, Trotter 1981)

The effect of timolol eye drops on intraocular pressure in cats has been studied, but results are not consistent. One study found reduction of IOP in untreated and treated eyes of healthy cats. Likewise, pupil diameter reduced, only in treated eyes. (Wilkie, Latimer 1991) When timolol was administered as a single drop once daily during eight days no significant or consistent reduction of IOP was found. However, pupil diameter was significantly smaller 4 to 8 hours after treatment, both in healthy cats and glaucomatous cats. In the same study no signs of ocular irritation or adverse effects of timolol were noticed and heart rates did not significantly alter. (Kiland 2016) On the contrary, another study found significantly reduced heart rates 20 minutes after timolol treatment. The median reduction of the heart rate was 25 bpm. (Gunther-Harrington 2016)

A third topical treatment option is the combination of brinzolamide and timolol. Although this combination has not been investigated in cats yet, it has been investigated in humans. Shin (2000) concluded that brinzolamide 1% administered three times daily (q8h) in combination with timolol 0.5% administered twice daily (q12h) reduced IOP significantly in humans with ocular hypertension. Besides, it was well tolerated and safe. (Shin 2000) Other studies found mean IOP reductions of 29.6% to 33.5% (Kaback, Scoper et al. 2008) and 28.4% to 34.9% (Manni, Denis et al. 2009) across all six on-therapy assessment times when brinzolamide 1% and timolol 0.5% eye drops both administered twice daily (q12h) in humans with open-angle glaucoma or ocular hypertension.

The aim of this study was to investigate the effects of brinzolamide 1% eye drops administered three times daily (q8h) in combination with timolol 0.5% eye drops administered two times daily (q12h) on the circadian rhythm of IOP, heart rate and pupil diameter in healthy cats.

Materials and methods

Animals

The twelve clinically healthy domestic shorthair cats of the colony of the Department of Clinical Sciences of Companion Animals, Faculty of Veterinary Medicine, Utrecht University entered the study. There were six male and six female cats and all were neutered. The cats were bred in Spain for clinical teaching purposes and around one and a half year old at the time of this study. The male and the female cats were separately group-housed and both groups had a little roofed space outside. The cats were fed once daily, around 8:30 AM. The light-phase started around 8 AM and ended around 8 PM. Ophthalmic examination by a board-certified veterinary ophthalmologist (Dip. ECVO) did not reveal any relevant abnormalities.

Study design

This study was a prospective, randomised, blinded study. The cats were divided in four groups by using the Random Team Generator. The four groups got all four treatments specified below by rotating the treatment protocols (Latin square). The eye that would receive treatment was randomly assigned by using the "flip a coin"-method.

First, measurements were performed on all cats for five days to obtain adjustment period values and for the examiners and cats to get used to the measurements. After one day of rest placebo measurements were obtained for five days, by administering artificial tears to one randomly assigned eye. Hereafter there was another day of rest, followed by the investigation of different treatment protocols. Every treatment period lasted five days and was alternated with three washout days.

IOP, horizontal pupil diameter, ambient light intensity at eye level and heart rate were measured q3h during the adjustment, placebo and treatment periods and during the last washout day. Immediately after eye drop administration the cats were observed for signs of ocular irritation. Measurements were mainly executed by four examiners split in two pairs and incidentally by two other examiners. This study was approved by the Authority of Animal Welfare Utrecht.

Medication

During the placebo period, artificial tear drops (Lacriforte, AST farma B.V., Oudewater, The Netherlands) were administered five times daily in the randomly assigned eye, at the same time points the medication would have been administered (7:30 and 7:45 AM, and 3:30, 7:30 and 11:30 PM).

Following the placebo period the cats were alternatingly exposed to one of four treatment protocols. These were: brinzolamide 1% eye drops q8h (Azopt, Alcon, Camberley, UK) and artificial tear drops q12h; dorzolamide 2% eye drops q8h (Dorzolamide 20 mg/ml, Cetrafarm B.V., Etten-Leur, The Netherlands) and artificial tear drops q12h; brinzolamide 1% eye drops q8h and timolol 0.5% eye drops q12h (Timolol Sandoz 5 mg/ml, Sandoz, Almere, The Netherlands); and dorzolamide 2% eye drops q8h + timolol 0.5% eye drops q12h. The contralateral eye received an artificial tear drop at all five medication time points.

During the medication periods brinzolamide 1% and dorzolamide 2% eye drops were administered three times daily (7:30 AM and 3:30 and 11:30 PM) and timolol 0.5% eye drops or artificial tear drops were administered two times daily (7:45 AM and 7:45 PM).

Measurements

IOP was measured using a rebound tonometer (TonoVet, ICare, Vantaa, Finland) as indicated by the manufacturer. From the adjustment period three successful IOP values of both eyes were obtained using the TonoVet, firstly in OD and secondly in OS (IOP1). Starting in the placebo period, immediately after the first set another set of three successful IOP values of both eyes was obtained, firstly in OD and secondly in OS (IOP2). The mean of the three values of either IOP1 or IOP2 was used for analysis. Heart rate was measured using a stethoscope (3M™ Littmann stethoscope, Classic II S.E. Stethoscope, Canada), horizontal pupil diameter using digital calipers positioned at 2 mm distance from the central cornea (kwb Germany GmbH, Stuhr, Germany) and light intensity at eye level using a luxmeter (Mastech, Luxmeter MS6610, Mastech Digital, Pittsburgh, Pennsylvania, USA). Ocular irritation was scored by the duration of the cats squeezing their eyes: not, five seconds, thirty seconds, more than thirty seconds or more than thirty seconds with extreme resistance.

The cats were measured in a constant order. IOP's, heart rates and pupil diameters were measured eight times daily (9 and 12 AM, 3, 6, 9 and 12 PM and 3 and 6 AM). Measurements were performed in an illuminated room with translucent curtains and light intensity at eye level was measured after every pupil diameter measurement. Every week the eyes of all cats were examined by a board-certified veterinary ophthalmologist (Dip. ECVO) for possible abnormalities, including signs of ocular irritation.

Data analysis

Statistical analysis was performed using R Studio (3.4.2). Linear mixed-effects models were used for data analysis of the IOP's of the treated and untreated eyes, pupil diameters of the treated and untreated eyes and heart rates. The tested factors in this models were IOP, pupil diameter, heart rate, treatment, day, time point, animal and treated eye, from which only the significant factors were used in the models. Factors were removed from the model if they decreased the AIC-value or if they increased the AIC-value maximal by two when they were left out from the model. Differences were considered significant if P-values <0.05 were found. Values are noted as mean ± SE.

Results

Unfortunately two cats, one male and one female, had to be removed from the study after the placebo measurements due to lack of cooperation during the administration of eye drops.

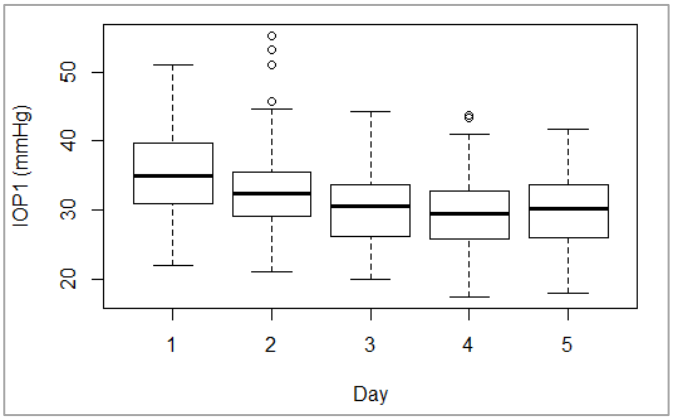
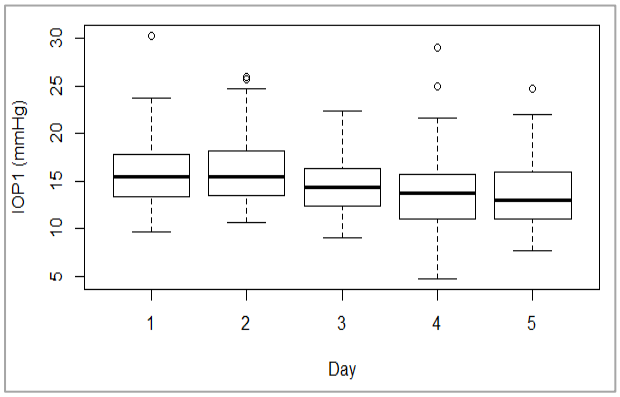


Figure 1: Boxplots of the mean intraocular pressure (IOP) on each day of the adjustment period in ten healthy cats.

Intraocular pressure

The mean IOP1 of all days of the adjustment period was 16.0 ± 0.21 mmHg and of the placebo period 14.9 ± 0.22 mmHg. This was a statistically significant difference ($P < 0.0001$). However, to compare adjustment period and placebo period only day 3, 4 and 5 were used, since data of day 1 and 2 of the adjustment period were not useful. Some values were missing since in the beginning the measurements required more time than available. Figure 1 shows that mean IOP values were increased with a higher variance during acclimatization to the measurements. On day 3, 4 and 5 IOP1 values stabilised.

Mean overall IOP1 of day 3, 4 and 5 of the adjustment period and the placebo period were respectively 15.2 ± 0.24 mmHg and 14.2 ± 0.26 mmHg, which was a statistically significant difference ($P = 0.0002$). However, differences were not significant at all time points. IOP1 in the placebo period was significantly lower than in the adjustment period at 9 AM and 3 and 12 PM.



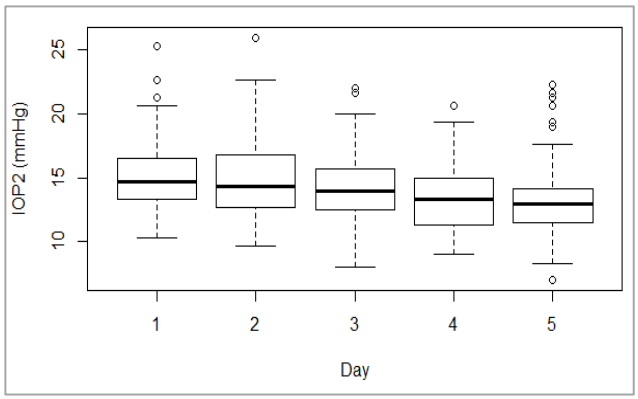


Figure 2 & 3: Boxplots of the mean intraocular pressures (IOP1 & IOP2) on each day of the placebo period in ten healthy cats. Notice the difference in scales.

For the following results the second set of IOP measurements was used for analysis (IOP2) since those values showed less variance and seemed less affected by increased sympathetic tone. All five days were used to compare placebo period, brinzolamide treatment period and brinzolamide and timolol treatment period. In addition treated and untreated eyes were compared. The mean IOP2 of all days of the brinzolamide and timolol treatment period was 11.3 ± 0.17 mmHg, a statistically significant reduction of 20% (2.9 mmHg) ($P < 0.0001$) as compared to the placebo period. At all time points the difference was statistically significant. The mean reduction was maximally 4.8 ± 0.46 mmHg (34%) at 9 AM and minimally 1.4 ± 0.47 mmHg (10%) at 3 PM. The mean IOP2 of all days of the brinzolamide treatment period was 11.9 ± 0.15 mmHg, which is 5% lower than IOP2 following brinzolamide eye drops alone. However, the reduction was not significant ($P = 0.30$). There were no significant differences at any time point or any day when comparing brinzolamide and timolol with brinzolamide alone.

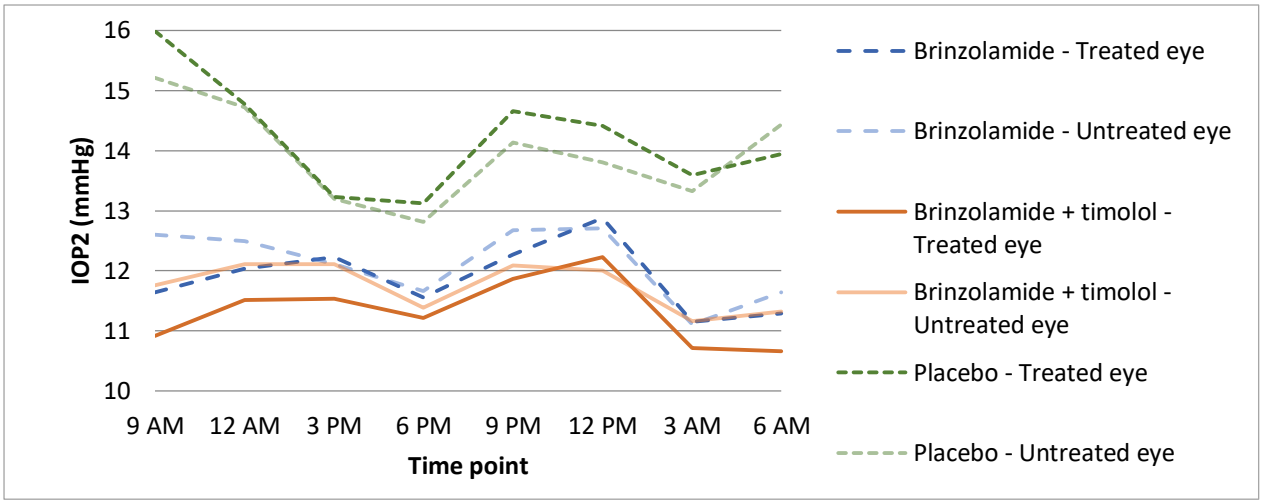


Figure 4: Mean intraocular pressure (IOP2) values at different time points during the day in the placebo, brinzolamide and brinzolamide + timolol treatment periods in ten healthy cats.

Figure 4 shows IOP2 in both treated and untreated eyes during placebo period, brinzolamide treatment period and brinzolamide and timolol treatment period. IOP2 during the placebo period showed most variations during a day and a circadian rhythm. The highest value was 16.0 ± 0.46 mmHg at 9 AM and the lowest value was 13.1 ± 0.47 mmHg at 6 PM. Another peak value was 14.7 ± 0.47 mmHg at 9 PM. After brinzolamide and brinzolamide and timolol treatment those peak values reduced. After brinzolamide and timolol treatment the highest value was 12.2 ± 0.54 mmHg at 12 PM and the lowest value was 10.7 ± 0.54 mmHg at 6 AM. The maximal difference during a day was then decreased with 48%, from 2.9 mmHg during the placebo period to 1.5 mmHg during the brinzolamide and timolol treatment period.

The IOP2 values of the untreated eyes during the brinzolamide and timolol (11.7 ± 0.17 mmHg) and brinzolamide alone (12.1 ± 0.17 mmHg) treatment periods were both statistically significant decreased as compared to the placebo period (14.0 ± 0.17 mmHg) ($P < 0.0001$ and $P < 0.0001$). The IOP2 values of the untreated eyes decreased with 16% during brinzolamide and timolol treatment and 14% during brinzolamide treatment as compared to the placebo period. The reductions were significant at all time points for both the brinzolamide and timolol and the brinzolamide alone treatment periods as compared to the placebo period.

There was no significant difference between treated eyes and untreated eyes during the brinzolamide and timolol treatment period. Only at 12 PM the mean IOP value of the treated eyes was higher than the mean IOP value of the untreated eyes. At 9 AM there was a statistically significant difference between the treated and untreated eyes of 0.8 ± 0.42 mmHg ($P = 0.044$). Likewise, the only statistically significant difference between the treated and untreated eyes during the brinzolamide treatment period was at 9 AM (1.0 ± 0.44 mmHg). During the placebo treatment period there were no significant differences between treated and untreated eyes at any time point.

Pupil diameter

The mean pupil diameters of day 3, 4 and 5 of adjustment period and placebo period were 4.8 ± 0.10 mm and 4.8 ± 0.10 mm and were not statistically different ($P = 0.43$). When time points were compared between adjustment period and placebo period, pupil diameters were significantly different at 9 AM and 12 AM. The differences were respectively -0.6 ± 0.24 mm and 0.9 ± 0.24 mm. The overall mean pupil diameters during placebo period, brinzolamide treatment period and brinzolamide and timolol treatment period were respectively 5.1 ± 0.08 mm, 5.4 ± 0.08 mm and 4.4 ± 0.08 mm. The

pupil diameters during the brinzolamide and timolol treatment period were significantly different as compared to the placebo period (P = 0.0002) and to the brinzolamide treatment period (P < 0.0001).

The pupil diameter during the brinzolamide and timolol treatment period was significantly smaller from 9 AM until 12 PM compared to the placebo period, with the biggest reduction at 9 PM of 1.3 ± 0.24 mm (19%). Mean pupil diameters after brinzolamide treatment did not significantly differ from mean pupil diameters after placebo treatment.

In addition, there was a significant difference between the pupil diameter of the untreated eyes during the placebo period (5.1 ± 0.09 mm) and during the brinzolamide and timolol treatment period (6.0 ± 0.09 mm) (P < 0.0001). At all time points the pupil diameter of the untreated eye during the brinzolamide and timolol treatment period was significantly larger than during the placebo period. Enlargement varied between 0.6 ± 0.20 mm at 9 PM and 1.7 ± 0.20 mm at 3 AM. Compared to the brinzolamide treatment period, the mean pupil diameters were significant larger at 12 AM, 3 PM and 3 and 6 AM. The highest increase was 0.6 ± 0.20 mm at 6 AM.

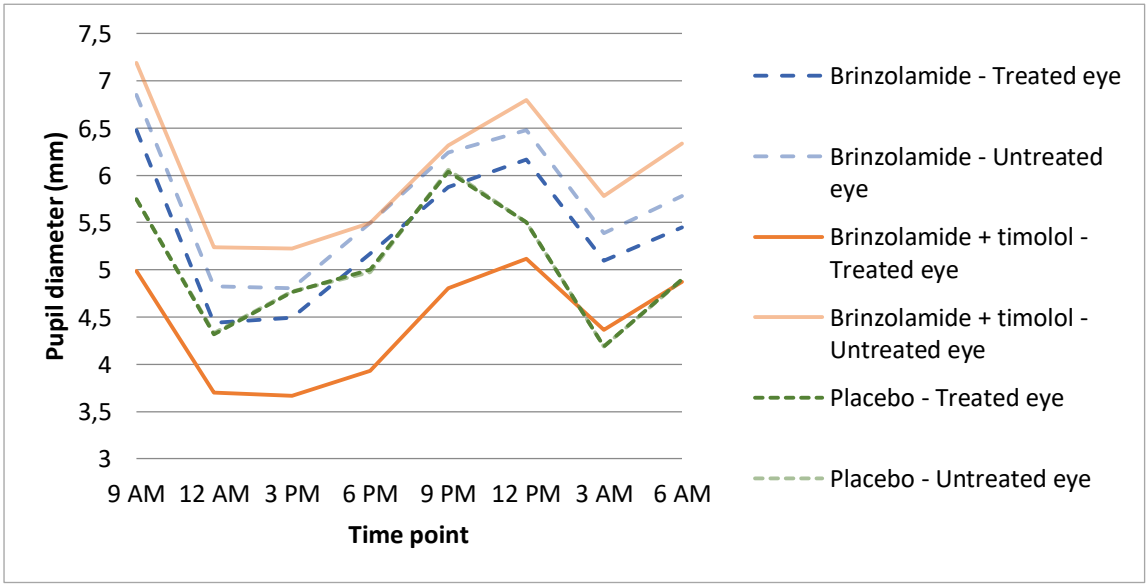


Figure 5: Mean horizontal pupil diameter in placebo, brinzolamide and brinzolamide + timolol treatment periods in ten healthy cats.

The mean light intensity during all measurements was 247 ± 1.7 lux, with a mean peak value of 345 lux and the highest variance at 12 AM. The mean values at 12 AM varied between days from 232 ± 48 lux to 734 ± 48 lux. Light intensities during day 3, 4 and 5 of the adjustment period and placebo period were significant different (P = 0.017), respectively 246 ± 5.7 lux and 232 ± 5.7 lux. Mean light intensities during the four treatment periods were all four significant different from the placebo period (P = 0.0002 – 0.021). The mean light intensity during the four treatment periods was 250 ± 5.4 lux and during the placebo period 235 ± 5.4 lux. Light intensity was a significant factor in the linear mixed-effects models of the pupil diameters of the treated and untreated eyes.

Heart rate

Heart rates showed a circadian rhythm during adjustment period and placebo period. During placebo period a difference of 30 ± 3.6 bpm was found between lowest values at 9 PM and peak values at 6 AM. Treatment with brinzolamide and timolol did not significantly alter mean overall heart rates,152 ± 1.3 bpm during the placebo period and 152 ± 1.3 bpm during the brinzolamide and timolol treatment period (P = 0.73). However, after brinzolamide and timolol treatment the circadian rhythm diminished

and varied 12 ± 2.8 bpm between 9 PM and 12 AM. No significant differences were found at any time point between brinzolamide treatment period and brinzolamide and timolol treatment period.

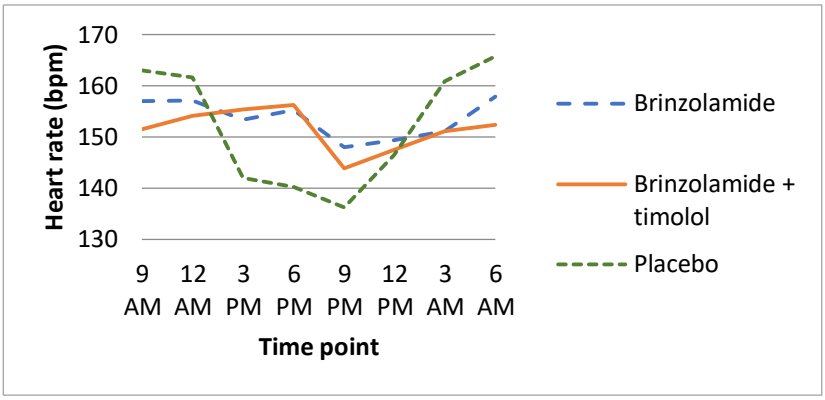


Figure 6: Mean heart rate at different time points of the day in the placebo, brinzolamide and brinzolamide + timolol treatment periods in ten healthy cats.

Ocular irritation

No ocular irritation was observed after artificial teardrop, brinzolamide eye drop or timolol eye drop administration. However, two of the twelve cats did not tolerate any eye drop administration. No for this research relevant abnormalities were found at the weekly examination of the eyes by the ophthalmology specialists.

Discussion

IOP of day 3, 4 and 5 of the adjustment period and the placebo period were statistically significantly different, respectively 15.2 ± 0.24 mmHg and 14.2 ± 0.26 mmHg. A statistically significant reduction of IOP of 20% was found during the brinzolamide and timolol treatment period compared to the placebo period. During a day the reduction of the IOP during the brinzolamide and timolol treatment period fluctuated between 10% and 34%.

In contrast to Gray (2003), in our study treatment with brinzolamide alone decreased IOP significantly in healthy cats. This can be explained by a difference in the frequency of eye drop administration, since in Gray (2003) eye drops were applied twice daily and in our study three times daily. In dogs (Gelatt, MacKay 2001) and humans (Silver 1998), administration of brinzolamide eye drops twice daily was sufficient for a significant reduction of IOP from baseline. Gray (2003) attributed the lack of IOP lowering effect of brinzolamide eye drops applied twice daily to the higher active aqueous humor production in cats as compared to that in dogs and humans (Gray 2003) and hypothesized that, when brinzolamide inhibits a certain amount of aqueous humor production per time, it has relatively less effect in cats. (Gray 2003)

We documented a circadian rhythm of IOP in cats. Unlike Sole (2007), who found peak values of 17.3 ± 0.4 mmHg at 12 AM and 20.5 ± 0.5 at 12 PM, we found peak values of 16.0 ± 0.46 mmHg at 9 AM and 14.7 ± 0.47 mmHg at 9 PM. In both studies the lowest value was at 6 PM, respectively 13.1 ± 0.47 mmHg (this study) and 15.8 ± 0.4 mmHg (Sole 2007). Explanations for the differences in time points of peak values may be a difference in the light and dark phases, feeding schedules and activity. In our study the peak value in the evening may be explained by the nocturnal behaviour of cats and the peak value in the morning by the feeding time around 8:30 AM. This causes a higher activity, which may lead to an increased IOP. (Sole 2007)

Simultaneous with IOP in the treated eye, IOP in the untreated eye decreased significantly following the topical application of either brinzolamide alone or brinzolamide and timolol. Possibly brinzolamide reached the contralateral eye through the systemic circulation. This assumption is further supported by the change in circadian heart rhythm in the brinzolamide and timolol treatment period. Since there were no significant differences in heart rate between brinzolamide treatment period and brinzolamide and timolol treatment period, the change in circadian heart rhythm may be an effect of

brinzolamide alone. Although systemic side effects of topically administered dorzolamide have been reported such as hypokalemia and renal tubular acidosis (Thiessen, Tofflemire et al. 2016), no such systemic effect of brinzolamide has been reported before in cats. In previous reports on topical brinzolamide administration in cats, however, the frequency of administration was lower than in our study. There were no clear indications of a systemic effect of timolol, since IOP and pupil diameter in untreated eyes were not significantly different from those following brinzolamide treatment alone. Likewise there were no significant differences between heart rate values with or without timolol. However, in Gunther-Harrington (2016) mean heart rates were significantly decreased 20 minutes after timolol eye drops application (Gunther-Harrington 2016).

Another explanation for the reduction in IOP of the untreated eye may be further acclimatization. This study assumed a week was sufficient for the cats to acclimatize. However, the cats may have been even more relaxed after the placebo period and therefore have reduced IOP in both eyes.

A disadvantage of timolol eye drops is, that they may be contraindicated in patients with uveitis, feline asthma or cardiac disease, because of inhibition of cardiac muscle activity and bronchodilation (McLellan 2011). Timolol, when applied unilaterally q12h for 5 days, caused no significant systemic effects in the ten healthy cats in our study. However, cats with feline asthma or cardiac disease may be more sensitive to beta blocking agents than healthy cats.

According to Kiland (2016), a clinically significant reduction in IOP is considered to be at least ~25% in cats with feline primary congenital glaucoma (Kiland 2016). In our study brinzolamide and timolol eye drops caused a mean reduction of IOP of 20.4% in treated eyes of healthy cats. The effect of brinzolamide 1% eye drops administered three times daily (q8h) and timolol 0.5% eye drops administered two times daily (q12h) is worth investigating in cats with glaucoma. The addition of timolol to the brinzolamide treatment may not be clinically relevant for lowering IOP since the difference in IOP of 5.0% as compared to treatment with brinzolamide alone was not significant. However, the effect may depend on the activity of the cat. Sympathetic adrenergic tone is required for timolol to reduce IOP (Colasanti, Trotter 1981). Denervation of the sympathetic nerves of cat eyes almost completely dissolved the effect of timolol. Therefore the IOP lowering effect of timolol is less efficient in sleeping cats than in active cats. (Colasanti, Trotter 1981, Kiland 2016, McLellan 2011) A benefit of timolol may be a neuroprotective effect. Since there were no clear indications of a systemic effect of timolol, timolol may not reach the optic nerve head which is necessary for the neuroprotective effect. Other methods for timolol to be delivered to the optic nerve head are worth investigating. (Osborne, Wood et al. 2005)

Two of the twelve cats in our study did not tolerate the administration of eye drops. This illustrates that topical medication may not be a suitable treatment for all cats. There will always be cats that require another treatment than eye drops.

Limitations of our study include possible falsely increased IOP or sympathetic tone, different examiners, persisting miosis and healthy cats. IOP may be falsely increased due to improper tonometer technique, inappropriate restraint or patient stress (McLellan 2011). Besides an increased IOP, patient stress may also result in increased pupil diameter and heart rate. During this research some falsely increased IOP measurements may have been obtained. The cats and the examiners were not acclimatized to the measurements prior to the study. Adjustment period was meant for the cats and examiners to get used to the measurements. In this period cats may have been more stressed and tonometer technique of the examiners may initially have been improper. Therefore part of the adjustment period values may not have been accurate and the values of the first two days of the

adjustment period were removed. Besides, since the 9 AM values of the first day of the placebo period were missing, values of 9 AM of the second day of the placebo period have been used. This seemed the most accurate solution. Most of the measurements were done by four examiners, split in two pairs. This may have resulted in slightly different results. Through the use of a Latin square and day and night shifts in the schedule for the examiners the possible influences were spread over the different treatments and time points.

Despite the cats being used to the measurements, there may have been other factors that have caused increased sympathetic tone in the cats. During day sometimes there were loud noises outside the research room, i.e. barking dogs, loud talking or cleaning sounds. Some cats may have been scared by those noises, resulting in stress and falsely increased IOP, pupil diameter and heart rate. During the third week of the research the cats started to sneeze, which lasted for about two weeks. The cats received different medications during the sneezing due to the Latin square design. The sneezing seems not related to a certain medication. An infection is the most likely cause, since it only lasted for two weeks and it has not been reported before in other articles. No other symptoms were found, but the sneezing might have influenced the results.

For practical reasons, eye drops were administered by the examiners. The medication bottles were taped and numbered, to reduce bias. However, treatments were not completely blinded since treated eye and treatment could partly be recognized from differences in bottles, time of administration and pupil diameter differences. When measurements are performed every three hours during 42 days it is not possible to have the measurements done by the same examiners every measurement throughout the study.

The washout period in this study was based on a study by Kiland (2016), who found that 12 hours after timolol treatment miosis of the treated eye was no longer significant. However, in a study by Wilkie, Latimer (1991) miosis persisted for up to a week after treatment. In both studies the values were obtained after one drop of 0.5% timolol in a randomly assigned eye. In our study miosis of the treated eye persisted during the three washout days, possibly as a result of accumulation of timolol in the treated eye. This may have affected results of the following treatment period. In Wilkie, Latimer (1991) IOP was significantly decreased 6, 8, 10 and 12 hours after treatment. Since in this study the IOP was only evaluated over 12 hours, IOP may possibly have been decreased for a longer time. (Wilkie, Latimer 1991) However, in Kiland (2016) IOP was only 4 hours after treatment significantly lower (Kiland 2016).

The cats used in this study are not representative for the population of glaucomatous cats. Since the cats used were healthy cats, IOP may have decreased less than it would have in glaucomatous cats (Gray 2003). Besides, the cats were around one and a half year old and most glaucomatous cats are older cats. As some of the cats were from the same litter, there was less variation in genomes. Furthermore, since the cats were housed in a small room with little space outside and since they were not very active, results may not be extrapolated to more active cats.

Conclusion

Brinzolamide 1% eye drops applied q8h in combination with timolol 0.5% eye drops applied q12h resulted in a significant mean 20% decrease in IOP in 10 healthy domestic shorthair cats. The combined administration of brinzolamide and timolol did not result in a significantly higher decrease in IOP than did brinzolamide administered q8h alone. In addition, a side effect of timolol was miosis and there were indications for a systemic effect of brinzolamide. Further investigation of the effect of brinzolamide and timolol in glaucomatous cats is necessary to define clinical significance in glaucomatous cats.

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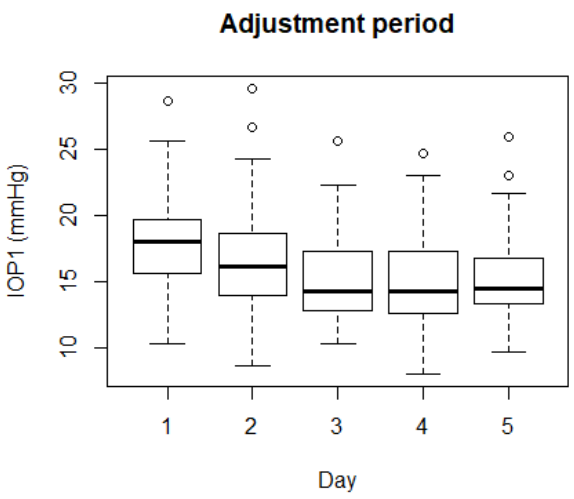
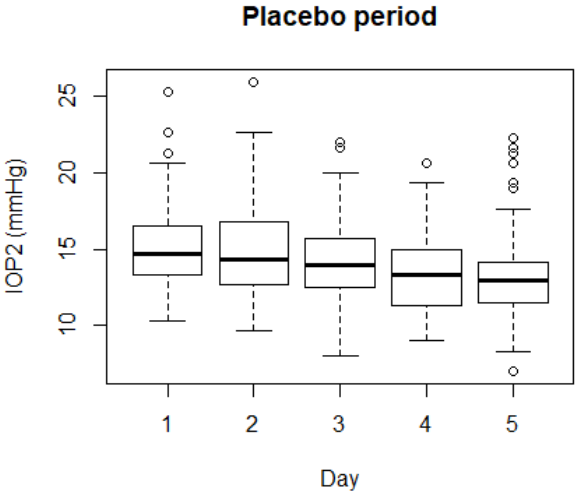
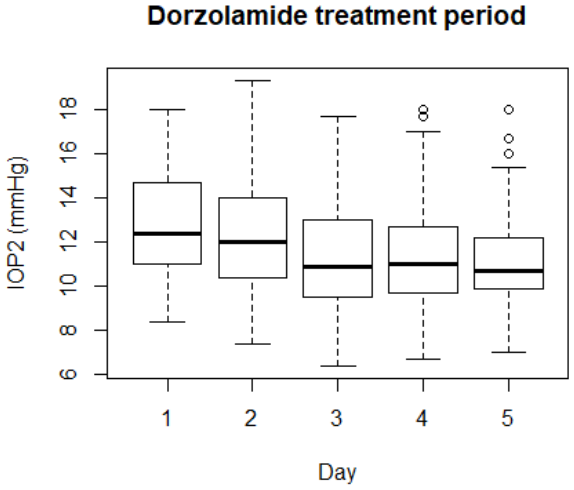
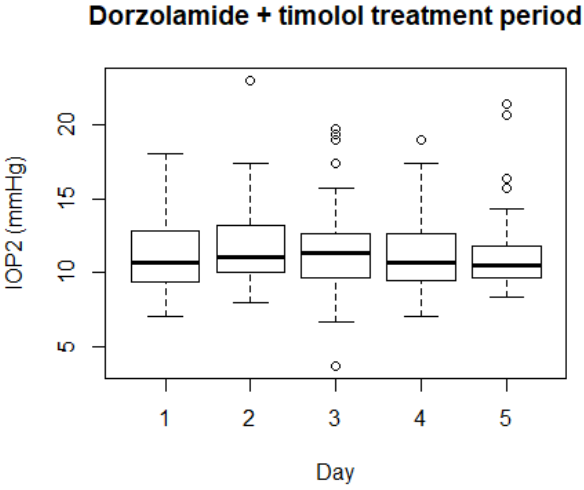
Attachments

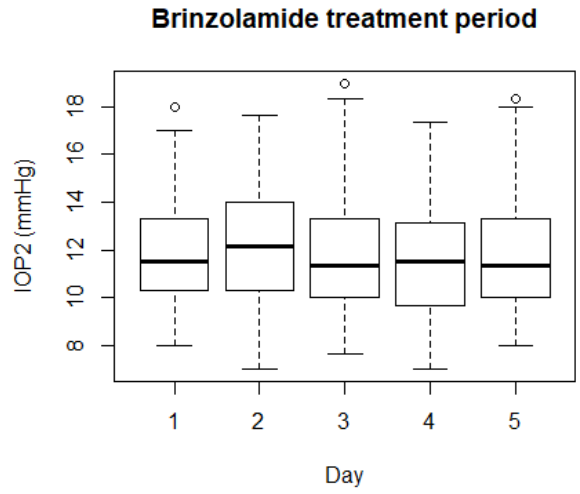
Boxplots of the mean IOP on each day of the adjustment, placebo and treatment periods in ten healthy cats.

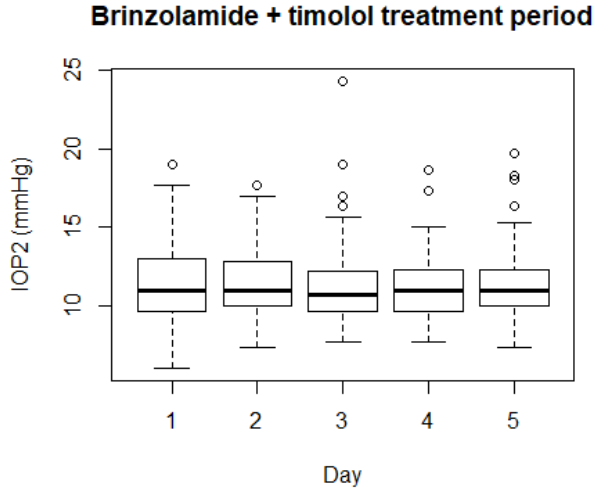




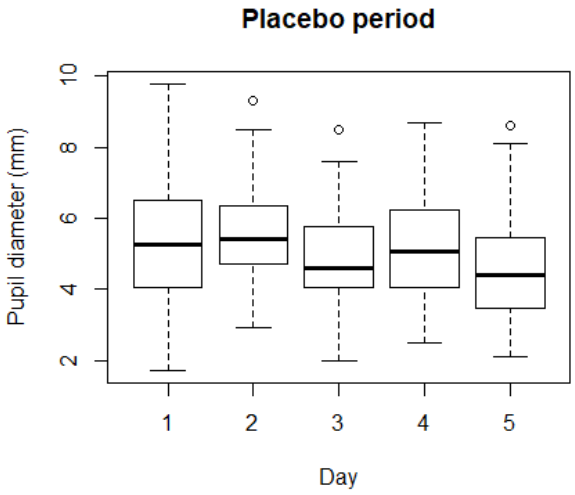


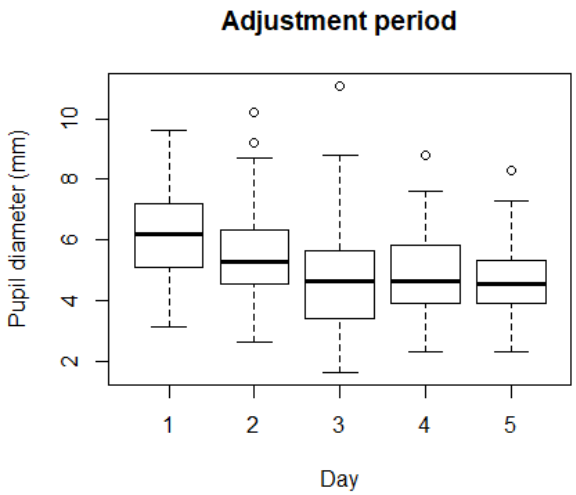


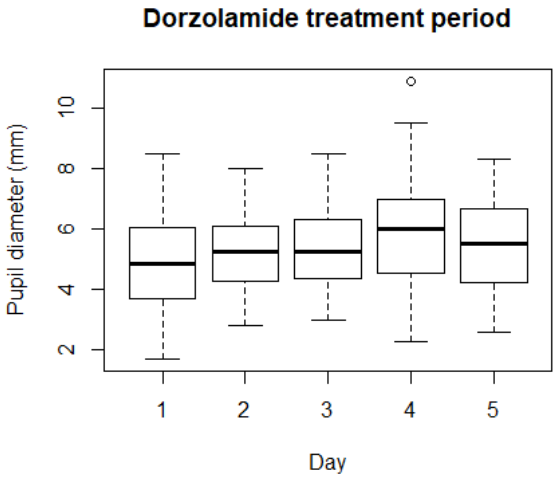


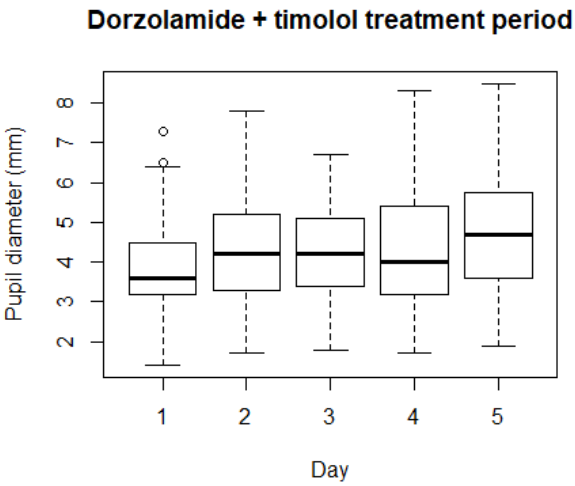


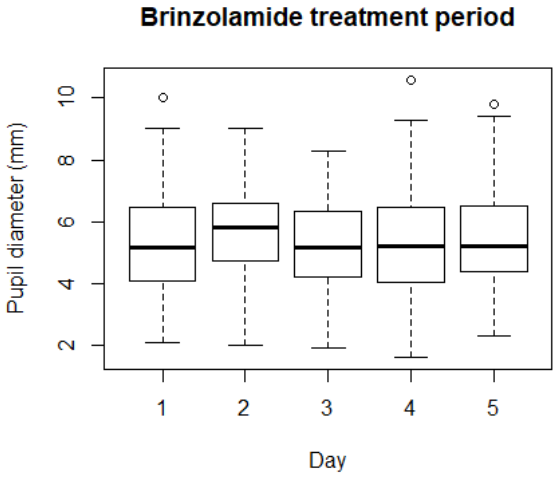
Boxplots of the pupil diameters on each day of the adjustment, placebo and treatment periods in ten healthy cats.

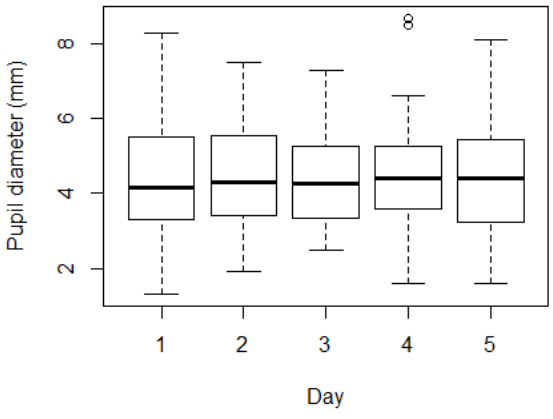




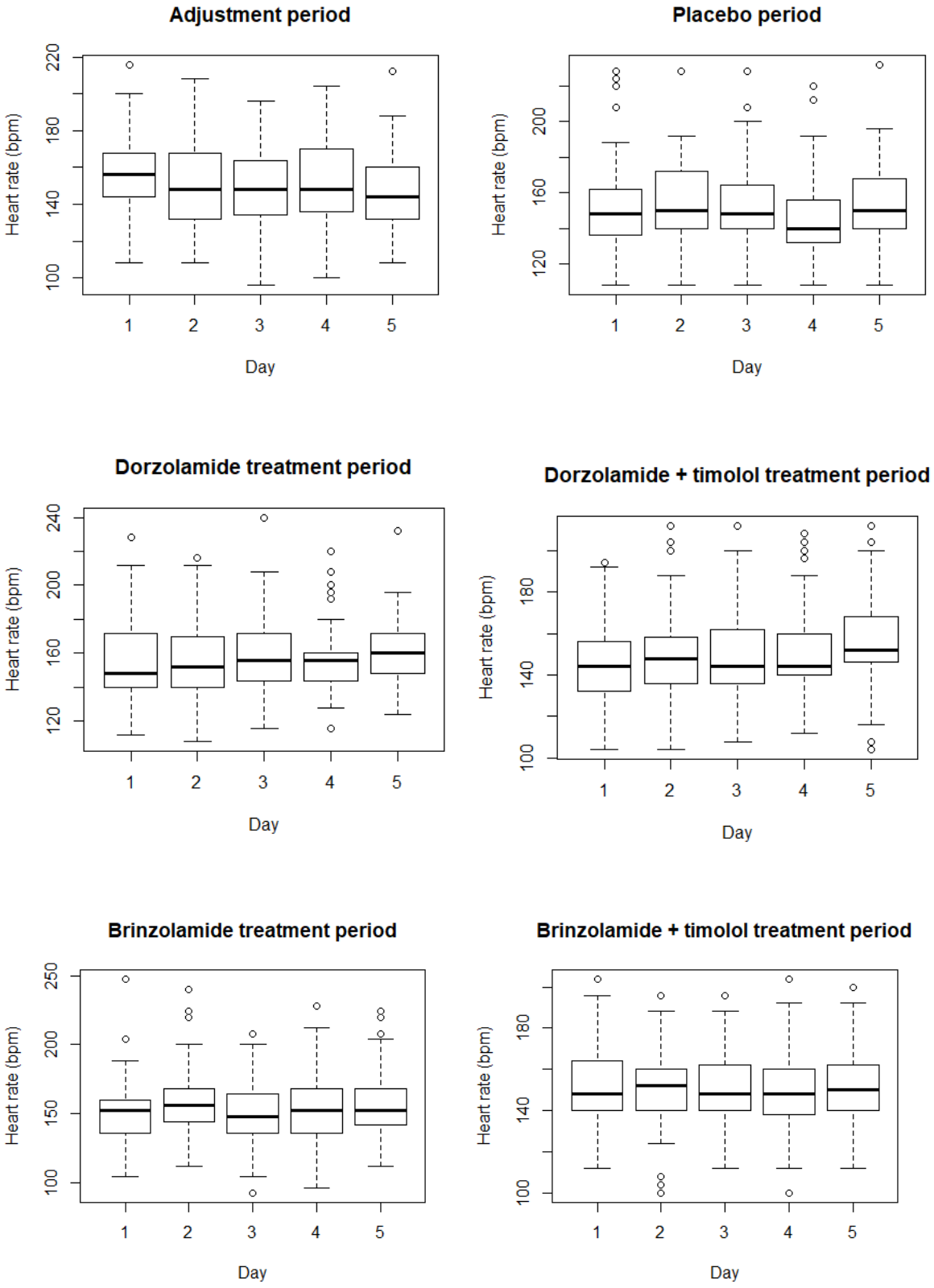








Boxplots of the heart rates on each day of the adjustment, placebo and treatment periods in ten healthy cats.



Tables of the results of the linear mixed-effects models of IOP, pupil diameter and heart rate in ten healthy cats.

Variable	Estimate/coefficient	Standard error	P-value
(Intercept)	13.77	0,98	-
Heart rate	0.0091	0.0038	0.0157
Pupil diameter	0.19	0.064	0.0029
OD/OS	0.12	0.76	0.8828
Brinzolamide			
9:00 AM	-4.44	0.45	-
12:00 AM	-2.72	0.44	-
3:00 PM	-0.96	0.45	0.0311
6:00 PM	-1.73	0.45	0.0001
9:00 PM	-2.47	0.45	-
12:00 PM	-1.68	0.44	0.0002
3:00 AM	-2.52	0.45	-
6:00 AM	-2.7	0.45	-
Brinzolamide + timolol			
9:00 AM	-4.83	0.45	-
12:00 AM	-3.07	0.44	-
3:00 PM	-1.51	0.45	0.0009
6:00 PM	-1.85	0.45	-
9:00 PM	-2.63	0.45	-
12:00 PM	-2.12	0.44	-
3:00 AM	-2.82	0.44	-
6:00 AM	-3.17	0.45	-

Table 1: Results of the linear effects-model of the intraocular pressure in the treated eyes in ten healthy cats. The placebo period was used as reference treatment.

Variable	Estimate/coefficient	Standard error	P-value
(Intercept)	6.12	0.49	-
Heart rate	-0.0014	0.0017	0.4137
IOP2	0.031	0.013	0.0212
Light intensity	-0.0019	0.0004	-
Brinzolamide			
Day 1	0.53	0.25	0.0374
Day 2	0.69	0.25	0.0059
Day 3	0.91	0.25	0.0003
Day 4	0.79	0.25	0.0015
Day 5	1.39	0.25	-
Brinzolamide + timolol			
Day 1	-0.84	0.26	0.0011
Day 2	-0.98	0.26	0.0001
Day 3	-0.54	0.25	0.0341
Day 4	-0.62	0.25	0.0136
Day 5	-0.11	0.25	0.6604
Brinzolamide			
9:00 AM	0.53	0.25	0.0374
12:00 AM	-0.17	0.25	0.5047
3:00 PM	-0.52	0.25	0.0359
6:00 PM	-0.039	0.25	0.8763
9:00 PM	-0.34	0.25	0.1771
12:00 PM	0.41	0.25	0.0993
3:00 AM	0.66	0.25	0.0081
6:00 AM	0.31	0.25	0.2227
Brinzolamide + timolol			
9:00 AM	-0.84	0.26	0.0011
12:00 AM	-0.81	0.25	0.0012
3:00 PM	-1.22	0.25	-
6:00 PM	-1.14	0.25	-
9:00 PM	-1.28	0.25	-
12:00 PM	-0.5	0.25	0.0443
3:00 AM	0.041	0.25	0.8688
6:00 AM	-0.13	0.25	0.6097

Table 2: Results of the linear effects-model of the pupil diameter in the treated eyes in ten healthy cats. The placebo period was used as reference treatment.

Variable	Estimate/coefficient	Standard error	P-value
(Intercept)	163.1	4.4	-
Brinzolamide			
9:00 AM	-6.2	3.4	0.0741
12:00 AM	-4.5	3.4	0.1939
3:00 PM	11.4	3.5	0.0010
6:00 PM	15.0	3.4	-
9:00 PM	12.1	3.5	0.0005
12:00 PM	2.8	3.5	0.4167
3:00 AM	-9.8	3.4	0.0047
6:00 AM	-7.6	3.5	0.0289
Brinzolamide + timolol			
9:00 AM	-11.6	3.4	0.0008
12:00 AM	-7.4	3.4	0.0311
3:00 PM	13.4	3.5	0.0001
6:00 PM	16.1	3.4	-
9:00 PM	7.6	3.4	0.0268
12:00 PM	1.0	3.4	0.7717
3:00 AM	-9.8	3.5	0.0047
6:00 AM	-13.2	3.5	0.0002

Table 3: Results of the linear effects-model of the heart rate in the treated eyes in ten healthy cats. The placebo period was used as reference treatment.