The effect of topically applied dorzolamide on intra-ocular pressure in healthy adult cats

Research Project Veterinary Medicine University Utrecht

4084926 R. de Gee

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Project tutors: drs. I.J.M. Slenter, dr. S.C. Djajadiningrat-Laanen

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Abstract

Objective: to investigate if dorzolamide 2% eye drops significantly lower intraocular pressure (IOP) in healthy adult cats.
Animals: nine 18-19 and one 12-month-old European shorthair cats (5 castrated males, 5 spayed females). All were healthy but one male cat which, during the study, was diagnosed with a mitral valve insufficiency.
Procedure: IOP, pupil diameter and heart rate were assessed with a rebound tonometer, digital callipers and stethoscope, respectively. All measurements were executed at 3-h intervals over 24-h periods spanning 42 days. These periods were divided into an adjustment phase (days 1-6), placebo phase (days 7-13) and treatment phase (days 14-42). Cats were divided into four groups (Random Team Generator). The first five days of the adjustment phase were used to get cats as well as their examiners accustomed to the procedures, and were followed by a day of rest. The placebo phase lasted five days followed by two days of rest. Then, five-day treatment periods were alternated with three-day washout periods. Apart from dorzolamide, three other kinds of medication (brinzolamide, dorzolamide with timolol and brinzolamide with timolol) were administered to the cats. These other kinds of medication are discussed in other papers. Each group of cats was rotated to a different kind of medication every eight days (Latin square). By the end of the study all cats had received all medications.
Results: A circadian rhythm in IOP during the adjustment and placebo phases was found as IOP was significantly different at 18:00, 21:00, 0:00, 3:00 and 6:00 in comparison with 9:00.
IOP following treatment with dorzolamide was significantly lower than following the placebo at all time points, and the variance between highest and lowest IOP during the day was smaller during the treatment phase. IOP in the untreated eye was not significantly different from the treated eye in the placebo and treatment phases. Pupil diameter and heart rate were not influenced by treatment with dorzolamide.
Conclusion: During treatment with dorzolamide, the daily fluctuations in IOP were less pronounced than during treatment with the placebo. Topical 2% dorzolamide significantly lowered IOP in healthy adult cats. Future research into dorzolamide as a possible treatment for glaucoma in cats is warranted.

Introduction

Background of the study

Glaucoma is a neurodegenerative disease of the retinal ganglion cells eventually resulting in loss of optic nerve fibres and blindness (1). Glaucoma is not a single disease entity, ‘glaucomas’ are a large, diverse group of disorders represented in all species. The most important feature of glaucoma in both humans and veterinary species is an elevated intra-ocular pressure (IOP) (1). However, elevated IOP does not necessarily mean glaucoma. The term for elevated IOP when there are no clinical signs of glaucomatous optic nerve and retinal damage is ocular hypertension. Both ocular hypertension and glaucoma should be distinguished from falsely increased IOP due to improper tonometric technique, inappropriate restraint, patient stress, and corneal factors that may render the tonometer inaccurate (1). In addition, IOP can fluctuate considerably within and between days, and therefore a single measurement of IOP can never be a definitive diagnosis for glaucoma without secondary

clinical signs. In advanced stages of glaucoma IOP may even be lowered, as degeneration of the ciliary body causes limitations on aqueous production. In such cases, the diagnosis of glaucoma relies on secondary characteristic abnormalities (1).
Normal IOP relies on a balance between aqueous outflow and the production of aqueous humour. In cats, most aqueous outflow (more than 97%) occurs from the posterior chamber through the pupil to the anterior chamber, exiting the eye in the trabecular meshwork and angular aqueous plexus. Then the fluid arrives in the intrascleral venous plexus and ultimately into general circulation (1). One of the most frequent causes of elevated IOP in cats is drainage angle obstruction by inflammatory debris or by post-inflammatory synechiae caused by uveitis, which lead to a reduction in aqueous outflow (2). Primary glaucoma in cats is rare (2, 3).
The only registered medication for glaucoma in cats is pilocarpine, a cholinergic miotic agent. Pilocarpine reduces IOP by increasing aqueous outflow. Blood-ocular barrier breakdown and miosis are adverse effects of pilocarpine. Therefore treatment with pilocarpine is contraindicated in patients who have uveitis in combination with glaucoma due to further pupil blockage (3). In humans, dorzolamide is a registered drug for glaucoma secondary to uveitis (4). This project focused on four potential treatments for glaucoma in cats, namely dorzolamide, brinzolamide, dorzolamide in combination with timolol, and brinzolamide in combination with timolol. In this paper only the effects of dorzolamide are discussed.

Mechanism of action of carbonic anhydrase inhibitors i.e. dorzolamide

The majority of aqueous fluid is produced by carbonic anhydrase (CA) isoenzymes. CA isoenzymes II, and to a lesser extent IV, are the most active in the ciliary body epithelium. CA isoenzymes are also found in erythrocytes and nephrons (1,3,4). CA isoenzymes catalyse a reversible reaction of hydration of carbon dioxide and dehydration of carbonic acid. In this process bicarbonate is created, which is excreted from the ciliary body epithelium into the posterior chamber of the eye creating an osmotic gradient. This process attracts water from the ciliary stromal vessels into the posterior chamber (1,3,4), resulting in aqueous humour formation.
Carbonic anhydrase inhibitors (CAIs) lower IOP by decreasing active aqueous humour production. CAIs add a sulphonamide group to the CA isoenzyme, competing with the attachment of carbonic acid and thereby suppressing the production of bicarbonate (1,3,4). Dorzolamide is a topically applied CAI selective for CA II and is considered a safe drug for humans (4). Topical glaucoma medication is preferred since systemic medication causes systemic inhibition of both CA II and IV and causes multiple adverse effects. These effects include diuresis, potassium depletion, tachypnea and metabolic acidosis. However, there is one case study that reported systemic adverse effects for dorzolamide when administered topically (5). Reported adverse effects of treatment with dorzolamide include transient salivation, inappetence and sterile conjunctivitis (3).

Objectives of the study

The goal of this study was to investigate if dorzolamide 2% eye drops significantly lower IOP in healthy adult cats.

Materials and methods

Animals

Twelve clinically healthy European shorthair cats (eleven 19-20 months old, one 12 months old, six castrated males, six neutered females), were included in this study. The cats were obtained and kept by the Faculty of Veterinary Medicine for educational purposes and will continued to be used for education after this project. All cats were group-housed with covered outside access to litterboxes. Male cats were separately housed from female cats. There was no automated light-dark cycle, the kennel was lit by artificial light and natural light. The caretakers of the kennel turned off the artificial light around 16:00-19:00, depending when the caretakers went home. Light was turned on around 8:00, when the cats were fed. The examiners briefly turned on the ambient kennel light when taking the cats to the examining room, although the light was not consistently turned off. The faculty dog kennel was located in the next room which was used for education, and as a result dogs were occasionally walked through the same kennel housing the cats.
This project was approved by the Animal Welfare Body Utrecht and overseen by a board-certified veterinary ophthalmologist (Dip. ECVO). The same board-certified veterinary ophthalmologist found one male cat with a circular red spot behind the left pupil, 3 female and 2 male cats with fine linear thickening around the nucleus. Light cornea oedema was also found with one of these 3 female cats. These ocular abnormalities in the cats were deemed not relevant to the project according to the expert opinion of the board-certified veterinary ophthalmologist.

Study design

This study was a prospective, randomised, and blinded research. The 12 cats were randomly divided into four groups (Random Team Generator). The medication was assigned to one eye which was randomly determined through the flip-a-coin method and an equal dose of artificial tears was applied to the fellow eye. The examiner was blinded to the medication. Four master's students divided in two teams with shifts every 12 hours collected the data, while receiving occasional assistance from the supervisors.
These periods were divided into an adjustment phase (days 1-6), placebo phase (days 7-13) and treatment phase (days 14-42).The first 5 days of the adjustment phase were used to get both cats and examiners accustomed to the procedures, followed by a day of rest. The placebo phase lasted 5 days followed by 2 days of rest. Then, 5-days' treatment periods were alternated with 3-days' washout periods. Only on the last day of each washout period measurements were performed. Apart from dorzolamide, three other kinds of medication (brinzolamide, dorzolamide with timolol and brinzolamide with timolol) were administered to the cats. Each group of cats was rotated to a different kind of medication every eight days (Latin square). By the end of the study all cats had received all medications.

Medication

No medication was given during the adjustment phase. During the placebo phase an artificial tear (Lacriforte, AST farma B.V., Oudewater, The Netherlands) was administered 5 times a day (7:30, 7:45, 15:30, 19:45 and 23:30) for 5 days. During each treatment phase the respective CAI was applied to the randomly selected eye at 7:30, 15:30, and 23:30 as well as timolol or an artificial tear on 7:45 and 19:45. The other eye was always administered an

artificial tear drop when the treatment eye was given any kind of teardrop. There were four protocols used in the treatment phase: A: Dorzolamide 2% eye drops 3dd (Dorzolamide 20 mg/ml, Cetrafarm B.V., Etten-Leur, The Netherlands) + artificial tear 2dd; B: Brinzolamide 1% eye drops (Azopt, Alcon, Camberley, UK) + artificial tear 2dd; C: Dorzolamide 2% 3dd + timolol 0,5% eye drops 2dd (Timolol Sandoz 5 mg/ml, Sandoz, Almere, The Netherlands); and D: Brinzolamide 1% 3dd + timolol 0,5% eye drops 2dd.

Measurements

For a valid interval measurement of this experiment, the following data per cat eye was collected at 3-hour intervals: IOP, pupil diameter, heart rate and light intensity on ocular level. The cats tolerated IOP measurements with gentle manual restraint in a sitting position and care was taken not to put pressure on the neck and eyelids. All cats were examined in the same order, starting with the queens and ending with the toms. Measurements were always started in the right eye, followed by the left eye.
A rebound tonometer (TonoVet, Icare, Vantaa, Finland) was used to measure the IOP. The tonometer was held in a 90° degree angle 1-4 millimetres from the cornea and for each eye three consecutive, valid IOP values were obtained ("IOP1"). For every IOP value a series of six individual measurements of IOP were obtained. The instrument discarded the highest and lowest result and calculated the mean from the middle four measurements. Only measurements with a deviation ≤1.8 mm Hg were used (no line shown on the display). When IOP varied more than 5 mm Hg in a set of measurements, the set was considered inaccurate and was repeated. Starting with the placebo phase, a second set of three consecutive IOP values ("IOP2") was obtained for the right and left eye. The time between the same measured eye for IOP1 and IOP2 was approximately one minute.
A stethoscope (Littmann Classic II SE, Medisafe LLP, Stratford, Conneticut, USA) was used to measure the heart rate. Digital calipers (kwb Germany GmbH, Stuhr, Germany) were used to measure horizontal pupil diameter. The calipers were held at a maximum distance of 2 mm to the cornea. A lux meter (Mastech luxmeter MS6610, Mastech Digital, Pittsburg, Pennsylvania, USA) was used to measure light intensity at eye level.

Statistical analysis

All collected data (IOP1, IOP2, heart rate, pupil diameter and light intensity) was analysed using a linear mixed-effects model (LME) computed using RStudio (6), with a referenced value (intercept) against fixed effects that were put into the model. A LME model makes an approximation of a function with a level of error. By adding categories to the LME model that are possible to control experimentally (fixed effects), and categories that are not possible to control experimentally (random effects) the amount of error is reduced. Fixed effects are controlled categories, for example: gender, type of treatment, or time of day.
The amount of error in a LME model is given structure by random effects. In the LME models used in this project the only random effect used is “subject”, the individual animal itself. This random effect is a unique trait of the animal due to individual differences.
The Akaike information criterion (AIC) is a parameter for loss of information in a statistical model, and as such is a parameter for the relative quality of a statistical model for a given dataset (7). A lower AIC means a better fit in a statistical model. Fixed effects were removed from the full model based on increased AIC values. 95% confidence interval sets were created using standard error computed by the model.

Eleven LME models were created: the last three days of the adjustment and placebo phase (model 1), all five days of the placebo and treatment phase for IOP1 (model 2) and IOP2 (model 3), the influence of the habituation on IOP in consecutive weeks for IOP1 (model 4) and IOP2 (model 5), both treated and untreated eyes measured during all five days of the placebo and treatment phase for IOP1 (model 6) and IOP2 (model 7), the light intensity (model 8), the pupil diameter in the treated eye (model 9) and untreated eye (model 10), and the heart rate (model 11). Fixed effects in the used models were: "time-measurement" and "gender" in model 1; "time-measurement" in models 2 and 3; "type of treatment", "time-measurement" and "week" in models 4 and 5; "eye of treatment" and "time-measurement" in models 6 and 7; "type of treatment" and "time-measurement" were used in models 8, 9, 10 and 11. Additional fixed effects were created using an interaction term, combining two fixed effects. An interaction term between "time-measurement" and "treatment" was created for models 1, 2, 3, 8, 9, 10 and 11. An interaction term between "week" and "type of treatment" was created for models 4 and 5. An interaction term between "eye of treatment" and "treatment" was created for models 5 and 6.
Visual inspection of residual plots in any of the used models did not reveal any obvious deviations from homoscedasticity or normality. The same cats were used in all models.

Results

Adjustment phase

It took time for the examiners to be well trained to collect the data in a correct way. The first two days of the adjustment phase were considered as unreliable data due to too many observed stress effects of the cats and because time differences were too great between measurements and therefore removed from the dataset. The medians and means with standard error of measurement (SEM) of the last three days of the adjustment phase calculated from all daily IOP measurements are shown in Table 1. A visual overview of the IOP in the adjustment phase (Z) is given in Figure 1. Medians and means of IOP measurements for the last three days of the adjustment phase differed not statistically significant (Table 1).

Table 1 – IOP1 (medians and means ± SEM) of the 5-days' adjustment phase including only the last three days of all 10 measured cats. All 8 different time points of measurements for IOP1 were included in the calculation.

	Day 3	Day 4	Day 5
Median IOP1	14.33	14.33	14.5
Mean IOP1	15.125 (0.441)	15.175 (0.441)	15.205 (0.441)

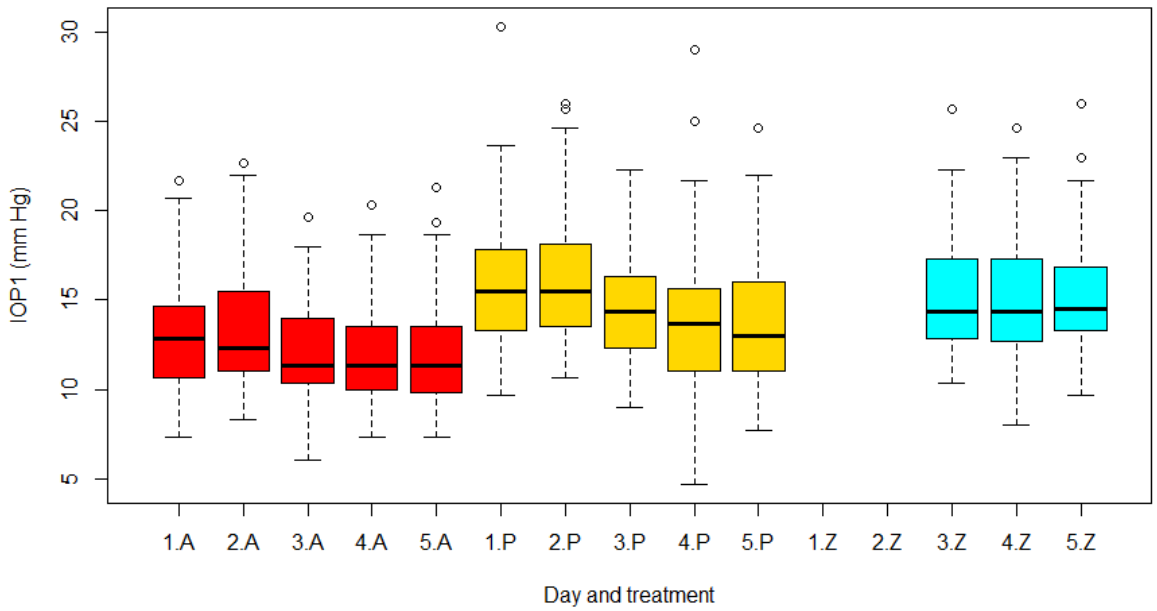

Figure 1 – Boxplot showing the overview of median and variance in IOP without treatment (Z), treatment with the placebo (P) and treatment with dorzolamide (A) in all 10 cats. All eight different time points of measurements for IOP1 were included in the calculation. Only the last three days of the adjustment phase (Z) were included. Extreme values extending across the first and fourth quadrants are shown as dots.

Placebo phase

Two cats, one male and one female, were removed from the project after the adjustment phase due to a serious lack of cooperation during the administration of eye drops. The collected data of the cats that were removed in the placebo phase were also removed from the entire dataset. The medians and means of IOP1 and IOP2 measurements of the placebo phase calculated from all daily IOP measurements are shown in Table 2. For both IOP1 and IOP 2, the first two days of the placebo phase had statistically significant higher medians and means than the last three days (Table 2). A visual overview of IOP1 in the placebo phase (P) is given in Figure 1.

Table 2 – IOP1 and IOP2 (medians and means ± SEM) of the 5-days' placebo phase of all 10 measured cats. All 8 different time points of measurements for IOP1 and IOP2 were included in the calculation.

	Day 1	Day 2	Day 3	Day 4	Day 5
Median IOP1	15.50	15.50	14.33	13.67	13.00
Mean IOP1	15.91 (0.445)	16.28 (0.445)	14.71 (0.445)	14.05 (0.445)	13.79 (0.445)
Median IOP2	14.67	14.33	14.00	13.33	13.00
Mean IOP2	15.25 (0.381)	15.00 (0.381)	14.11 (0.381)	13.41 (0.381)	13.33 (0.381)

Treatment phase

All ten cats tolerated topical application of medication and no ocular adverse effects other than anisocoria from treatment with timolol were observed during this study. The medians and means of IOP1 and IOP2 measurements of the treatment phase calculated from all daily IOP measurements are shown in Table 3. For both IOP1 and IOP 2, the first two days of the placebo phase had statistically significant higher medians and means than the last three days (Table 3). A visual overview of IOP1 in the treatment phase (A) is given in Figure 1.

Table 3 - IOP1 and IOP2 (medians and means ± SEM) of the 5-days' treatment phase of all 10 measured cats. All 8 different time points of measurements for IOP1 and IOP2 were included in the calculation.

	Day 1	Day 2	Day 3	Day 4	Day 5
Median IOP1	12.84	12.33	11.33	11.33	11.33
Mean IOP1	13.00 (0.399)	13.50 (0.399)	12.09 (0.399)	11.90 (0.399)	11.90 (0.405)
Median IOP2	12.33	12.00	10.84	11.00	10.70
Mean IOP2	12.69 (0.331)	12.44 (0.331)	11.33 (0.331)	11.31 (0.331)	11.09 (0.331)

Comparison of IOP1 in adjustment and placebo phases

According to the Faculty of Veterinary Medicine statistician, if zero is included in the range of the 95% confidence interval, the difference between a fixed effect and the intercept is not statistically significant. Difference in IOP without treatment and treatment with the placebo on 0:00, 6:00, 9:00, 15:00 and 18:00 are statistically insignificant (Table 4).
A circadian rhythm was found, with the highest and lowest IOP measurements being at 9:00 and 18:00 respectively which is visually shown in Figures 2 and 3. There are notable differences between median and mean on time points 0:00 and 6:00 (Figures 2 and 3).

Table 4 – 95% confidence intervals of IOP in the last three days without treatment (Z) and treatment with the placebo (P).

** The intercept values estimate IOP with lower and upper standard error at 9:00 without any treatment (Z) for a tomcat.*
*** Time point values are to be seen as the IOP difference from the intercept.*
**** To adjust for queens the female estimate value needs to be added to the respective value.*
***** The effect of the placebo is to be seen as the IOP difference at corresponding time points between the adjustment phase (Z) and placebo phase (P), for example: 12:00 P differs from 12:00 Z, 15:00 P differs from 15:00 Z and so on. 9:00 P differs from the intercept.*

IOP1				**95% Confidence interval**	
Fixed effects	**Estimate difference (mm Hg)**	**SEM**	**P value**	Lower bound	Upper Bound
Intercept*	16.91	0.805	0.00	15.35	18.46
12:00 Z**	-2.17	0.688	0.00	-3.49	-0.84
15:00 Z	-2.88	0.688	0.00	-4.20	-1.55
18:00 Z	-4.45	0.688	0.00	-5.87	-3.21
21:00 Z	-3.21	0.688	0.00	-4.54	-1.88
0:00 Z	-2.93	0.688	0.00	-4.26	-1.60
3:00 Z	-3.75	0.688	0.00	-5.08	-2.42
6:00 Z	-3.97	0.688	0.00	-5.29	-2.64
Female***	2.38	0.923	0.03	0.29	4.47
9:00 P****	-2.89	0.688	0.00	-4.22	-1.56
12:00 P	-0.90	0.688	0.19	-2.23	0.43
15:00 P	-1.94	0.688	0.00	-3.27	-0.62
18:00 P	-0.88	0.688	0.20	-2.21	0.45
21:00 P	0.05	0.688	0.95	-1.28	1.37
0:00 P	-1.40	0.688	0.04	-2.73	-0.07
3:00 P	-0.44	0.688	0.52	-1.77	0.88
6:00 P	0.51	0.688	0.46	-0.82	1.84

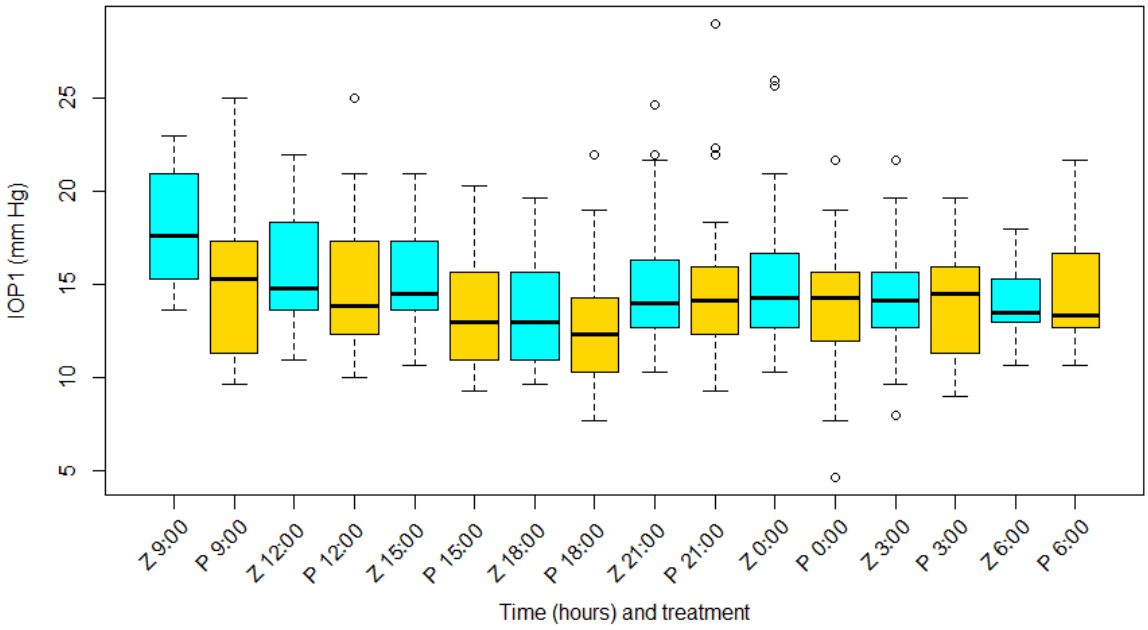

Figure 2 - Boxplot of the IOP without treatment (Z) and treatment with the placebo (P) in all 10 cats. Only the last 3 days of measurement for IOP1 were included in the calculation. Extreme values extending across the first and fourth quadrants are shown as dots.

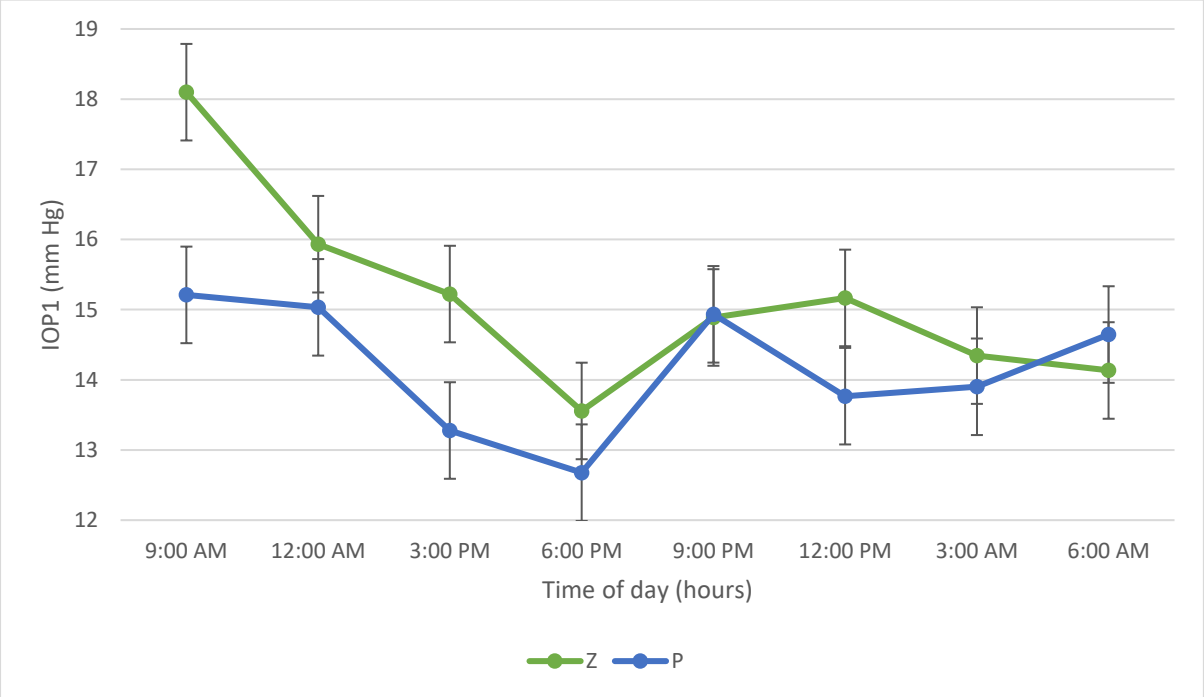

Figure 3 – Means of the IOP without treatment (Z) and treatment with the placebo (P) in all 10 cats with standard error (0.688 on all points). Only the last 3 days of measurement for IOP1 were included in the calculation. X-axis: time of day (hours), Y-axis: IOP (mm Hg).

Comparison of IOP1 and IOP2 in placebo and treatment phases

Following treatment with dorzolamide, IOP1 was significantly lower at all time points compared to treatment with a placebo (Table 5).

Table 5 – 95% confidence intervals of IOP1 in the last three days of the treatment phase (A) and placebo phase (P).

**The intercept values estimate IOP with lower and upper standard error at 9:00 with dorzolamide treatment (A) for a tomcat.*
*** Time point values are to be seen as the IOP difference from the intercept.*
**** To adjust for queens the female estimate value needs to be added to the respective value time point value.*
***** The effect of the placebo is to be seen as the IOP difference at corresponding time points between the treatment phase (A) and placebo phase (P), for example: 12:00 P differs from 12:00 A, 15:00 P differs from 15:00 A and so on. 9:00 P differs from the intercept.*

IOP1				**Confidence interval**	
Fixed effects	**Estimate difference (mm Hg)**	**SEM**	**P value**	Lower bound	Upper Bound
Intercept*	12.37	0.714	0.00	10.99	13.76
12:00 A**	-0.95	0.562	0.09	-2.04	0.14
15:00 A	-0.67	0.562	0.23	-1.77	0.42
18:00 A	-2.00	0.562	0.00	-3.09	-0.91
21:00 A	-0.25	0.565	0.66	-1.35	0.84
0:00 A	-0.75	0.565	0.19	-1.85	0.35
3:00 A	-2.01	0.565	0.00	-3.10	-0.91
6:00 A	-0.96	0.565	0.09	-2.06	0.14
Female***	2.20	0.851	0.03	0.26	4.14
9:00 P****	2.29	0.562	0.00	1.20	3.38
12:00 P	3.73	0.562	0.00	2.63	4.82
15:00 P	1.36	0.562	0.02	0.27	2.45
18:00 P	1.79	0.562	0.00	0.70	2.88
21:00 P	2.61	0.565	0.00	1.51	3.70
0:00 P	2.17	0.565	0.00	1.07	3.27
3:00 P	2.55	0.565	0.00	1.46	3.65
6:00 P	2.15	0.565	0.00	1.05	3.24

Following treatment with dorzolamide, the circadian rhythm levelled as compared to the placebo for IOP1 (Figures 4 and 5). This levelling can also be determined by the number of statistically significant differences within a phase. These changes occurred at 6:00 and 15:00 for treatment with dorzolamide and at 3:00, 6:00, 15:00 and 18:00 for treatment with the placebo (Table 5).
Medians and means were lowered when treated with dorzolamide as compared to the placebo (Figures 4 and 5). The mean difference in IOP1 for all time points between treatment with dorzolamide and the placebo was 2.33 mm Hg, as calculated from Table 5.

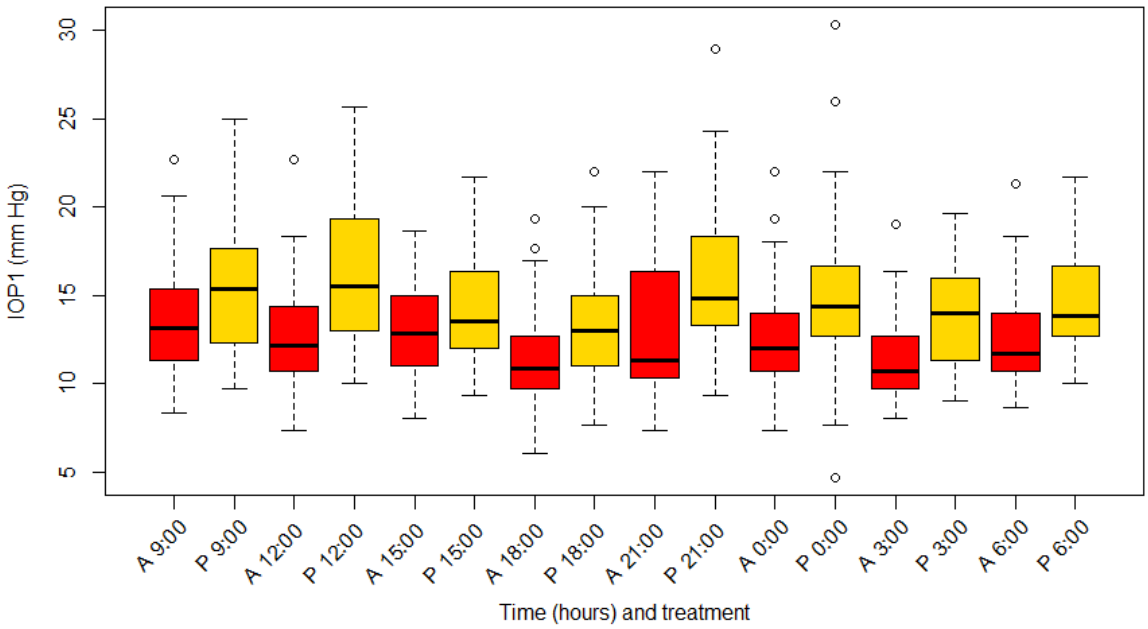

Figure 4 - Boxplot of the IOP to the treatment with dorzolamide (A) and the placebo (P) in all 10 cats. All 5 days of measurement for IOP1 were included in the calculation. Extreme values extending the first and fourth quadrant are shown as dots.

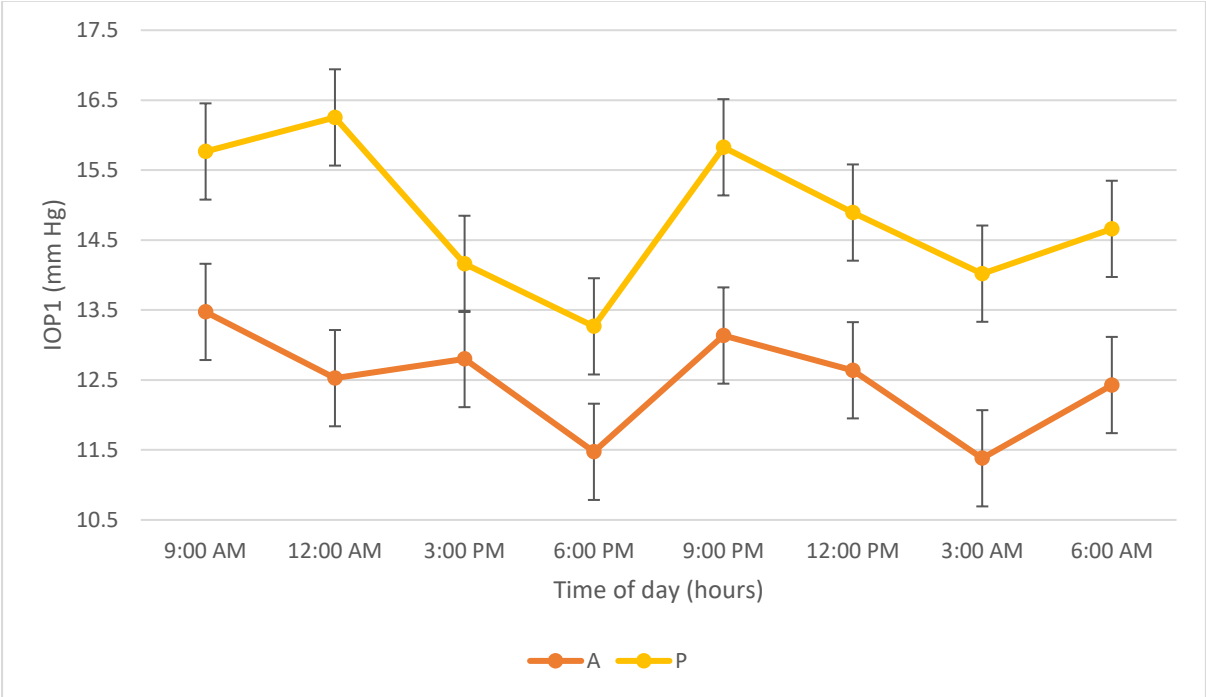

Figure 5 - Means of the IOP for treatment with dorzolamide (A) and placebo (P) in all 10 cats with standard error (0.56 on all points). All 5 days of measurement for IOP1 were included in the calculation. X-axis: time of day (hours), Y-axis: IOP (mm Hg).

Just like IOP1, the measurements of IOP2 were significantly lower on all time points for treatment with dorzolamide compared to treatment with a placebo (Table 6).

Table 6 - 95% confidence intervals of IOP2 in the last three days of the treatment phase (A) and placebo phase (P).

**The intercept values estimate IOP with lower and upper standard error at 9:00 with dorzolamide treatment (A) for a tomcat.*
*** Time point values are to be seen as the IOP difference from the intercept.*
**** To adjust for queens the female estimate value needs to be added to the respective value time point value.*
***** The effect of the placebo is to be seen as the IOP difference at corresponding time points between the treatment phase (A) and placebo phase (P), for example: 12:00 P differs from 12:00 A, 15:00 P differs from 15:00 A and so on. 9:00 P differs from the intercept.*

IOP2				**Confidence interval**	
Fixed effects	**Estimate difference (mm Hg)**	**SEM**	**P value**	Lower bound	Upper Bound
Intercept*	11.28	0.544	0.00	10.23	12.34
12:00 A**	-0.12	0.471	0.80	-1.04	0.80
15:00 A	-0.10	0.471	0.83	-1.02	0.82
18:00 A	-1.15	0.471	0.01	-2.07	-0.24
21:00 A	0.47	0.474	0.32	-0.45	1.39
0:00 A	0.02	0.474	0.97	-0.90	0.94
3:00 A	-1.00	0.474	0.04	-1.92	-0.08
6:00 A	-0.45	0.474	0.34	-1.37	0.47
Female***	1.63	0.619	0.03	0.22	3.04
9:00 P****	3.21	0.471	0.00	2.30	4.13
12:00 P	2.79	0.471	0.00	1.88	3.71
15:00 P	1.23	0.471	0.01	0.32	2.15
18:00 P	2.18	0.471	0.00	1.26	3.09
21:00 P	2.09	0.474	0.00	1.17	3.01
0:00 P	2.30	0.474	0.00	1.38	3.22
3:00 P	2.49	0.474	0.00	1.57	3.41
6:00 P	2.30	0.474	0.00	1.38	3.22

IOP2 has lower medians and means than IOP1 (Figure 6 and 7). The mean difference of IOP2 for all time points between treatment with dorzolamide and the placebo was 3.21 mm Hg, as calculated from Table 6.
Like IOP1, a levelling of the circadian rhythm occurred when treated with dorzolamide for IOP2 (Figure 6 and 7).

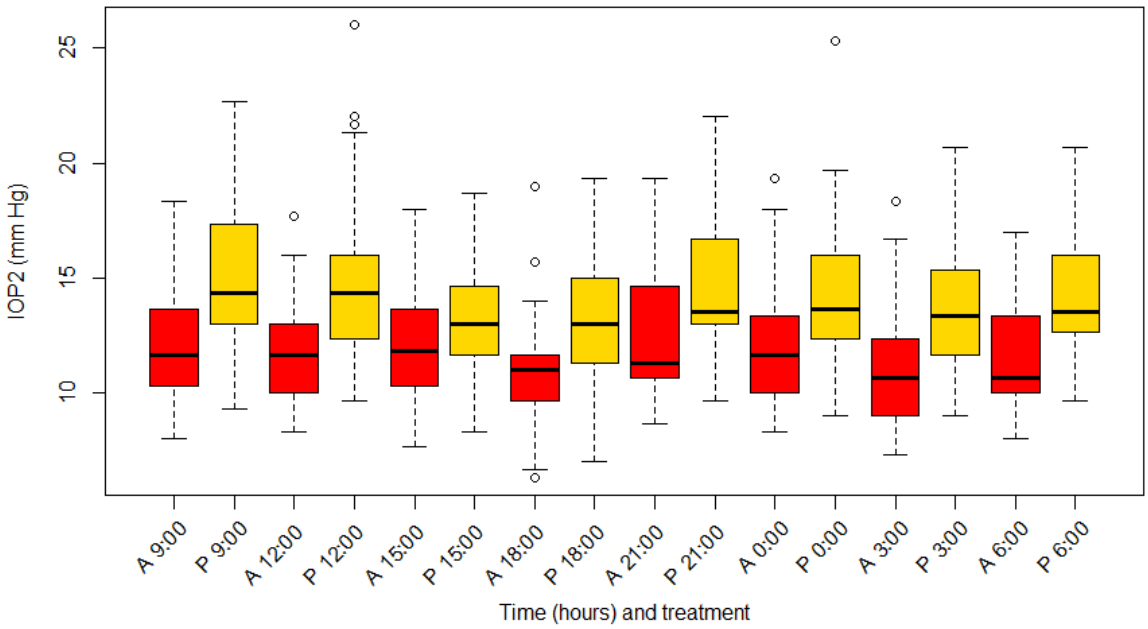

Figure 6 - Boxplot of the IOP to the treatment with dorzolamide (A) and the placebo (P) in all 10 cats. All 5 days of measurement for IOP2 were included in the calculation. Extreme values extending the first and fourth quadrant are shown as dots.

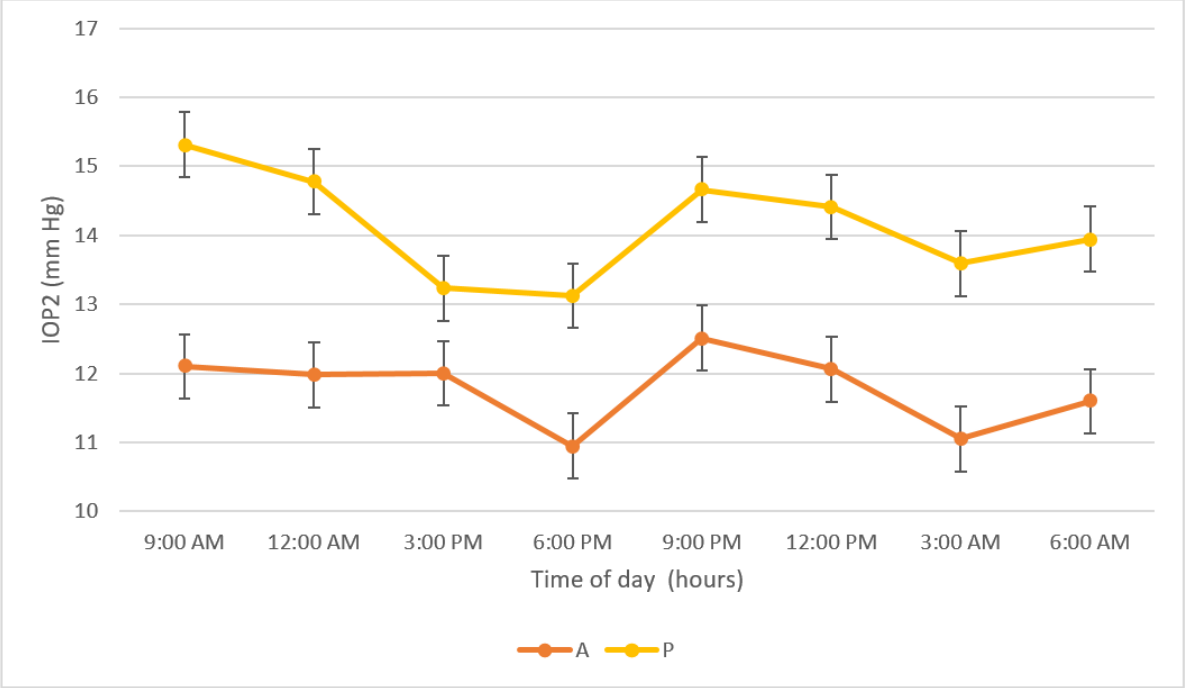

Figure 7 - Means of the IOP for treatment with dorzolamide (A) and placebo (P) in all 10 cats with standard error (0.47 on all points). All 5 days of measurement for IOP2 were included in the calculation. X-axis: time of day (hours), Y-axis: IOP (mm Hg).

The influence of the habituation of the research protocol was tested by comparing the IOP data of all treatments (dorzolamide, brinzolamide, dorzolamide in combination with timolol and brinzolamide in combination with timolol) over time (Figure 8 and 9). This was investigated by comparing consecutive weeks (Tables 7 and 8). There were no statistically significant differences found.

Table 7 - 95% confidence intervals of IOP1 of all different treatments of the entire project (A: dorzolamide, B: brinzolamide, C: dorzolamide in combination with timolol, D: brinzolamide in combination with timolol) across all four weeks of treatment (week 3 to 6).

** The intercept values estimate IOP with lower and upper standard error at 9:00 in week 3 with dorzolamide treatment (A).*
*** The effect of different treatments are to be seen as the difference between dorzolamide (A) (the intercept) and the respective treatment (B, C or D) on week 3.*
**** Time points are a fixed effect in this model. All time points are to be seen as difference in IOP of the intercept.*
***** The effect of the week is to be seen as the difference in IOP between week 3 of treatment with dorzolamide (A), e.g. the intercept.*
****** The effect of the week in different treatments is to be seen as the difference in IOP between treatment with dorzolamide (A) on the corresponding week and the respective treatment (B, C or D) on the same corresponding week, for example: Treatment B week 4 differs from Treatment A week 4, Treatment B week 5 differs from Treatment A week 5 and so on.*

IOP1				**Confidence interval**	
Fixed effects	**Estimate difference (mm Hg)**	**SEM**	**P value**	Lower bound	Upper Bound
Intercept*	12.36	0.557	0.00	11.27	13.44
Treatment B week 3**	-0.08	0.856	0.92	-1.75	1.58
Treatment C week 3	-0.27	0.613	0.66	-1.46	0.93
Treatment D week 3	-0.71	0.978	0.47	-2.62	1.19
12:00***	-0.11	0.245	0.65	-0.37	0.59
15:00	0.07	0.245	0.77	-0.41	0.55
18:00	-0.40	0.245	0.10	-0.88	0.08
21:00	0.84	0.245	0.00	0.36	1.32
00:00	0.94	0.242	0.00	0.47	1.41
03:00	-0.82	0.242	0.00	-1.29	-0.35
06:00	-0.46	0.242	0.06	-0.94	0.01
Treatment A Week 4****	0.70	0.973	0.47	-1.19	2.59
Treatment A Week 5	0.70	0.609	0.25	-0.48	1.89
Treatment A Week 6	-0.72	0.852	0.39	-2.38	0.94
Treatment B week 4*****	0.08	1.48	0.96	-2.80	2.96
Treatment C week 4	-1.05	1.216	0.39	-3.41	1.32
Treatment D week 4	0.08	1.896	0.97	-3.61	3.77
Treatment B week 5	0.01	1.217	0.99	-2.36	2.38
Treatment C week 5	-0.80	1.043	0.44	-2.83	1.23

Treatment D week 5	-0.59	1.219	0.63	-2.97	1.78
Treatment B week 6	0.43	1.66	0.80	-2.81	3.66
Treatment C week 6	0.66	1.216	0.59	-1.70	3.03
Treatment D week 6	1.58	1.481	0.29	-1.30	4.46

Table 8 - 95% confidence intervals of IOP2 of all different treatments of the entire project (A: dorzolamide, B: brinzolamide, C: dorzolamide in combination with timolol, D: brinzolamide in combination with timolol) across all four weeks of treatment (week 3 to 6).

** The intercept values estimate IOP with lower and upper standard error at 9:00 in week 3 with dorzolamide treatment (A).*
*** The effect of different treatments are the differences in IOP between dorzolamide (A) and the respective treatment (B, C or D) on week 3.*
**** Time points are a fixed effect in this model. All time points are to be seen as difference in IOP of the intercept.*
***** The effect of the week is to be seen as the difference in IOP between week 3 of treatment with dorzolamide (A).*
****** The effect of the week in different treatments is to be seen as the difference in IOP between treatment with dorzolamide (A) on the corresponding week and the respective treatment (B, C or D) on the same corresponding week, for example: Treatment B week 4 differs from Treatment A week 4, Treatment B week 5 differs from Treatment A week 5 and so on.*

IOP2				**Confidence interval**	
Fixed effects	**Estimate difference (mm Hg)**	**SEM**	**P value**	Lower bound	Upper Bound
Intercept*	11.57	0.549	0.00	10.5	12.64
Treatment B week 3**	-0.27	0.852	0.75	-1.93	1.39
Treatment C week 3	-1.03	0.540	0.06	-2.08	0.02
Treatment D week 3	-0.25	0.975	0.80	-2.15	1.65
12:00***	0.38	0.214	0.08	-0.04	0.80
15:00	0.40	0.214	0.06	-0.01	0.82
18:00	-0.16	0.214	0.46	-0.57	0.26
21:00	0.97	0.214	0.00	0.55	1.39
00:00	1.15	0.211	0.00	0.74	1.56
03:00	-0.37	0.211	0.08	-0.79	0.04
06:00	-0.02	0.211	0.93	-0.43	0.39
Treatment A week 4****	0.75	0.971	0.44	-1.14	2.63
Treatment A week 5	-0.11	0.537	0.83	-1.16	0.93
Treatment A week 6	-1.02	0.849	0.23	-2.67	0.63
Treatment B week 4*****	-0.12	1.478	0.93	-3.00	2.75
Treatment C week 4	-0.45	1.181	0.70	-2.75	1.85
Treatment D week 4	-0.96	1.903	0.61	-4.66	2.75
Treatment B week 5	0.55	1.182	0.64	-1.75	2.85

Treatment C week 5	0.45	0.921	0.62	-1.34	2.25
Treatment D week 5	-0.52	1.183	0.66	-2.83	1.78
Treatment B week 6	0.94	1.665	0.57	-2.30	4.18
Treatment C week 6	2.42	1.181	0.04	0.12	4.72
Treatment D week 6	0.65	1.479	0.66	-2.23	3.53

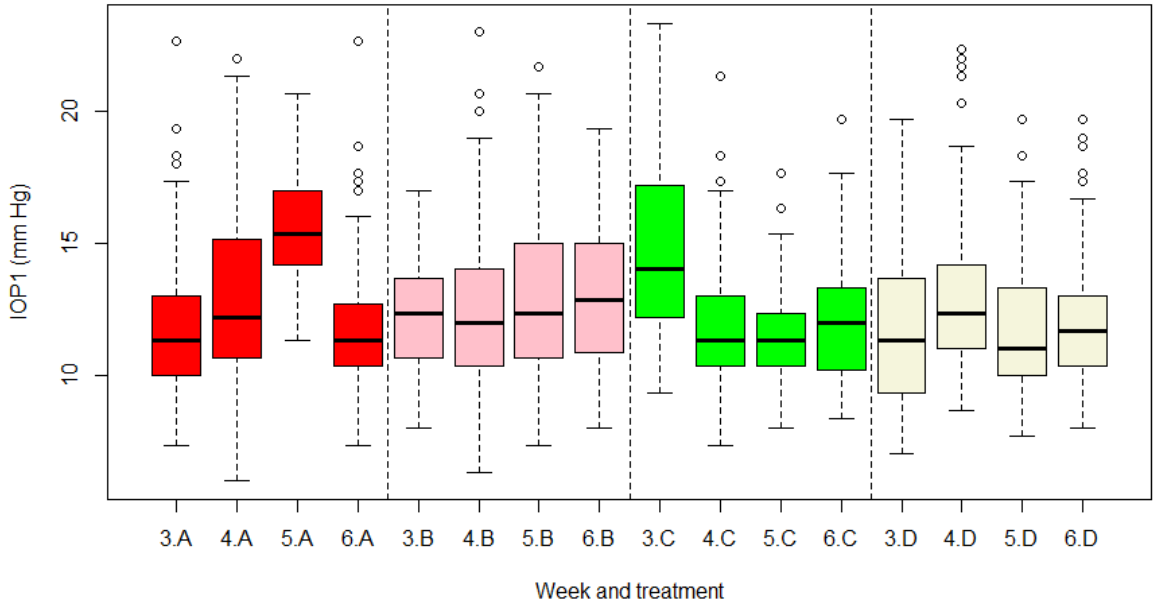

Figure 8 – Boxplot of the IOP to the treatment with dorzolamide (A), brinzolamide (B), dorzolamide in combination with timolol (C) and brinzolamide in combination with timolol (D) during all four weeks of treatment (week 3 to 6). All 5 days of measurement for IOP1 in each week were included in the calculation. Extreme values extending the first and fourth quadrant are shown as dots.

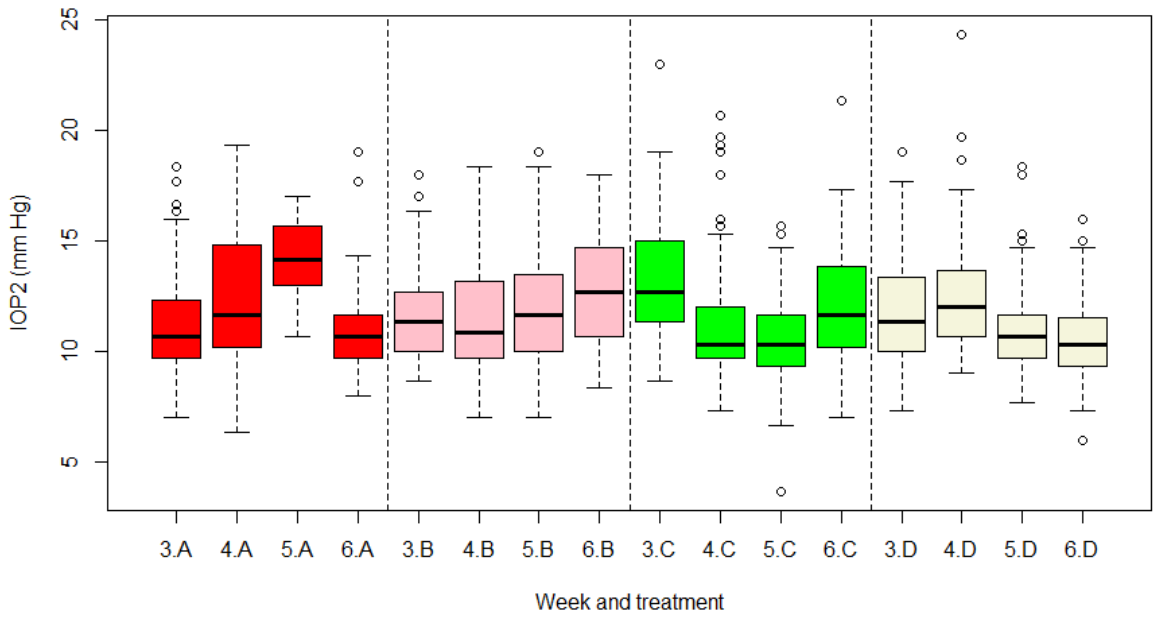

Figure 9 - Boxplot of the IOP to the treatment with dorzolamide (A), brinzolamide (B), dorzolamide in combination with timolol (C) and brinzolamide in combination with timolol (D) during all four weeks of treatment (week 3 to 6). All 5 days of measurement for IOP2 in each week were included in the calculation. Extreme values extending the first and fourth quadrant are shown as dots.

To test the difference between treated and untreated eyes, both eyes from all cats were compared to each other. There are no statistically significant differences between measurements of the treated eye to the untreated eye in the treatment phase and placebo phase, for IOP1 (Table 9) and IOP2 (Table 10). The median of the IOP ratios of treated and untreated eyes on all measured days is 1.029 for the placebo and 1.026 for treatment with dorzolamide for IOP1 (Figure 10) and 1.022 for the placebo and 1.024 for treatment with dorzolamide for IOP2 (Figure 11).

Table 9 - 95% confidence intervals of IOP1 in the treatment phase (A) and placebo phase (P) using both treated and untreated eyes.

** The intercept values estimate IOP with lower and upper standard error at 9:00 for the treated eye with dorzolamide treatment (A).*
*** Untreated eyes in the treatment phase with dorzolamide (A) are to be seen as difference from the intercept.*
**** Time points are a fixed effect in this model. All time points are to be seen as difference from the intercept.*
***** The effect of the placebo (P) is to be seen as the difference between the corresponding eye of treatment and treatment with dorzolamide (A). Treated P differs from the intercept and Untreated P differs from Untreated A.*

IOP1				**Confidence interval**	
Fixed effects	**Estimate difference (mm Hg)**	**SEM**	**P value**	Lower bound	Upper Bound
Intercept*	13.55	0.512	0.00	12.55	14.55
Untreated A**	-0.31	0.208	0.14	-0.71	0.10
12:00***	-0.39	0.293	0.18	-0.96	0.18
15:00	-1.11	0.293	0.00	-1.68	-0.53
18:00	-2.15	0.293	0.00	-2.72	-1.57
21:00	-0.32	0.293	0.28	-0.89	0.26

0:00	-1.03	0.293	0.00	-1.60	-0.45
3:00	-2.15	0.293	0.00	-2.72	-1.58
6:00	-1.15	0.293	0.00	-1.74	-0.57
Treated P* **	2.34	0.208	0.00	1.94	2.75
Untreated P	2.38	0.208	0.00	1.98	2.79

Table 10 - 95% confidence intervals of IOP2 in the treatment phase (A) and placebo phase (P) using both treated and untreated eyes.

** The intercept values estimate IOP with lower and upper standard error at 9:00 for the treated eye with dorzolamide treatment (A).*
*** Untreated eyes in the treatment phase with dorzolamide (A) are to be seen as difference from the intercept.*
**** Time points are a fixed effect in this model. All time points are to be seen as difference from the intercept.*
***** The effect of the placebo (P) is to be seen as the difference between the corresponding eye of treatment and treatment with dorzolamide (A). Treated P differs from the intercept and Untreated P differs from Untreated A.*

IOP2				**Confidence interval**	
Fixed effects	**Estimate difference (mm Hg)**	**SEM**	**P value**	Lower bound	Upper Bound
Intercept*	12.67	0.479	0.00	11.74	13.61
Untreated A**	-0.19	0.177	0.28	-0.53	0.16
12:00***	-0.35	0.248	0.16	-0.83	0.14
15:00	-1.12	0.248	0.00	-1.60	-0.63
18:00	-1.90	0.248	0.00	-2.38	-1.41
21:00	-0.33	0.248	0.19	-0.81	0.16
0:00	-0.76	0.248	0.00	-1.25	-0.28
3:00	-1.58	0.248	0.00	-2.07	-1.10
6:00	-0.93	0.248	0.00	-1.42	-0.44
Treated P****	2.34	0.176	0.00	2.00	2.69
Untreated P	2.32	0.176	0.00	1.98	2.67

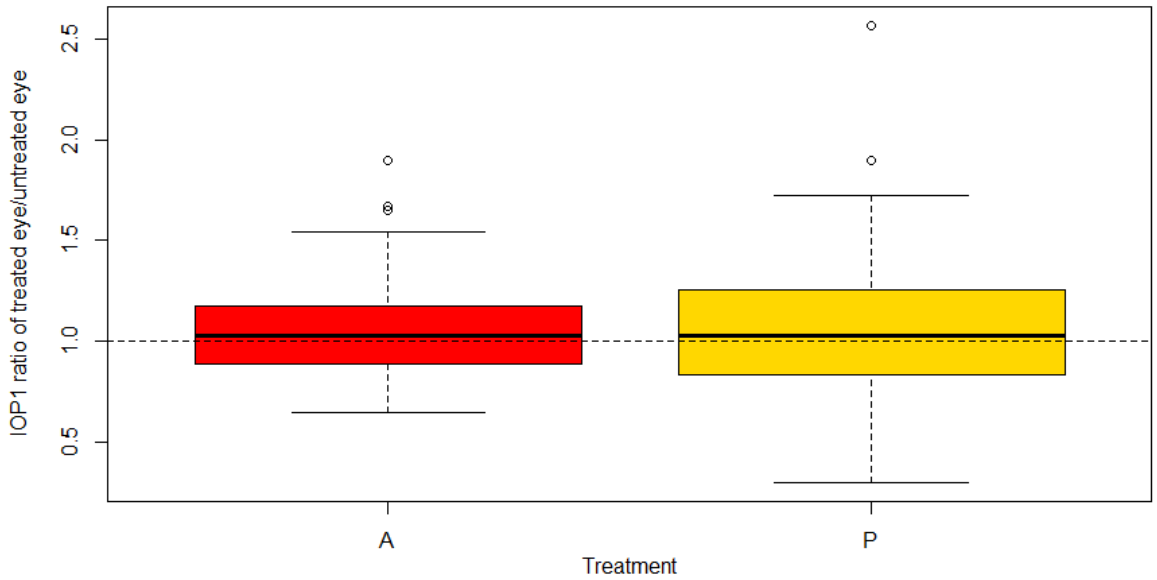

Figure 10 - Boxplot of the ratio of the IOP in the treated eye to the IOP in the untreated eye during dorzolamide (A) and placebo (P) treatment respectively. All 5 days of measurement for IOP1 were included in the calculation. Extreme values extending the first and fourth quadrant are shown as dots.

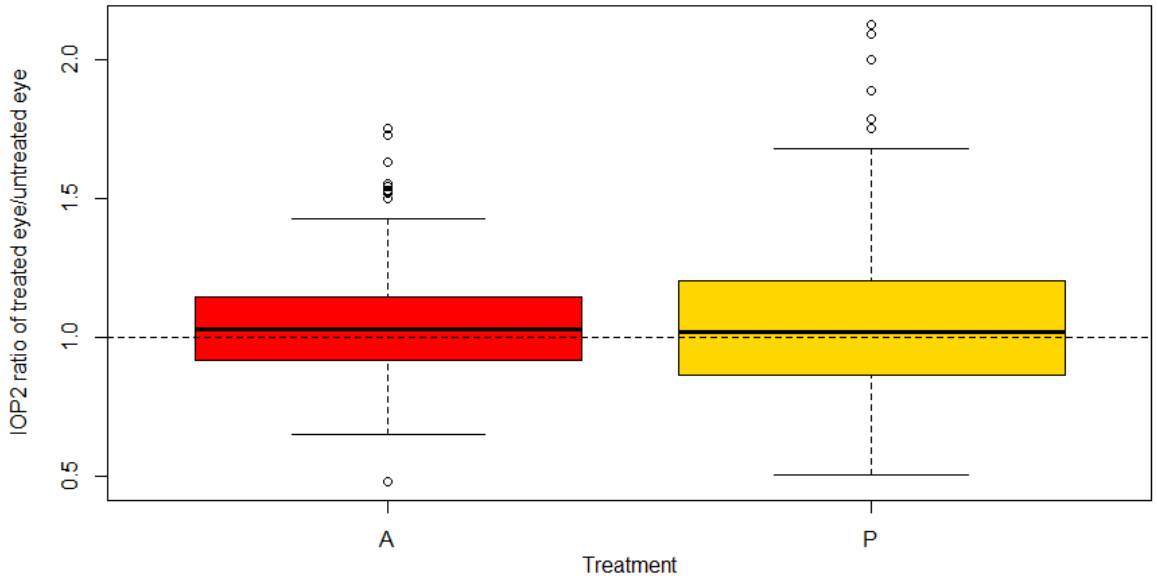

Figure 11 - Boxplot of the ratio of the IOP in the treated eye to the IOP in the untreated eye during dorzolamide (A) and placebo (P) treatment respectively. All 5 days of measurement for IOP2 were included in the calculation. Extreme values extending the first and fourth quadrant are shown as dots.

Comparison of light intensity, pupil diameter and heart rate

The light intensity between the treated eyes and untreated eyes were close to one (Table 11). Therefore a mean light intensity was used for comparison with the pupil diameter. Light intensity peaked at 12:00 (Table 12). Also the smallest pupil diameter during daytime was measured at 12:00 (Tables 13 and 14). A reduction in the pupil diameter was observed in treated eyes after treatment with timolol. Only the untreated eye differed statistically significant in the treatment phase (Table 13 and 14). Treated and untreated eyes showed high variance in pupil diameter (Figures 12 and 13).
The heart rate did not differ statistically significant when treated with dorzolamide as compared to treatment with the placebo or without treatment for all measured time points (Table 15) and showed high variance (Figure 14).

Table 11 – Ratio of the light intensity of the treated eye to the light intensity of the untreated eye during the treatment phase with dorzolamide, the placebo phase and the adjustment phase with no treatment respectively at all measured time points. All 5 days of measurement for the light intensity were included in the calculation for the treatment phase and placebo phase. Only the last 3 days of the adjustment phase were included.

Time of day / Type of treatment	9:00	12:00	15:00	18:00	21:00	0:00	3:00	6:00
Treatment phase	1.04	1.12	1.03	1.05	1.00	0.97	0.99	0.97
Placebo phase	1.00	1.15	0.99	1.04	1.03	1.03	1.04	1.03
Adjustment phase	0.99	1.08	1.02	1.05	1.05	1.01	1.00	1.01

Table 12 - 95% confidence intervals of the mean light intensity for both eyes without treatment (Z), treatment with the placebo (P) and treatment with dorzolamide (A).

** The intercept values estimate the light intensity with lower and upper standard error at 9:00 for placebo treatment (P).*
*** Treatment with the placebo (P) on different time points is to be seen as a difference in light intensity from the intercept.*
**** The effect of treatment with dorzolamide (A) and no treatment (Z) is to be seen as the light intensity difference at corresponding time points between treatment with the placebo (P) to dorzolamide (A) or no treatment (Z), for example: 12:00 A differs from 12:00 P, 12:00 Z differs from 12:00 P and so on. 9:00 A and 9:00 Z differ from the intercept.*

Light intensity				**Confidence interval**	
Fixed effects	**Estimate difference (lux)**	**SEM**	**P value**	Lower bound	Upper Bound
Intercept*	243.00	9.125	0.00	225.30	260.70
12:00 P**	117.44	12.261	0.00	93.66	141.22
15:00 P	-9.80	12.261	0.42	-33.58	13.98
18:00 P	-42.30	12.261	0.00	-66.08	-18.52
21:00 P	-51.90	12.261	0.00	-75.68	-28.12
0:00 P	-28.30	12.261	0.02	-52.08	-4.52
3:00 P	-24.94	12.261	0.04	-48.72	-1.16
6:00 P	-22.88	12.261	0.06	-46.66	0.90
9:00 A***	7.07	12.261	0.56	-16.71	30.85
12:00 A	-20.64	12.261	0.09	-44.42	3.14
15:00 A	26.43	12.261	0.03	2.65	50.21

18:00 A	41.59	12.261	0.00	17.81	65.37
21:00 A	30.42	12.323	0.01	6.52	54.32
0:00 A	18.82	12.323	0.13	-5.08	42.72
3:00 A	0.80	12.323	0.95	-23.10	24.70
6:00 A	2.78	12.323	0.82	-21.12	26.68
9:00 Z	-4.62	14.157	0.74	-32.07	22.84
12:00 Z	-46.67	14.157	0.00	-74.13	-19.22
15:00 Z	49.05	14.157	0.00	21.59	76.51
18:00 Z	30.77	14.157	0.03	3.31	58.22
21:00 Z	32.57	14.157	0.02	5.11	60.02
0:00 Z	3.02	14.157	0.83	-24.44	30.47
3:00 Z	13.89	14.157	0.33	-13.57	41.35
6:00 Z	6.13	14.157	0.67	-21.33	33.59

Table 13 - 95% confidence intervals of the horizontal pupil diameter of the eye of treatment without treatment (Z), treatment with the placebo (P) and treatment with dorzolamide (A).

** The intercept values estimate the pupil diameter with lower and upper standard error at 9:00 for placebo treatment (P).*
*** Treatment with the placebo (P) on different time points is to be seen as a difference in pupil diameter from the intercept.*
**** The effect of treatment with dorzolamide (A) and no treatment (Z) is to be seen as the pupil diameter difference at corresponding time points between treatment with the placebo (P) to dorzolamide (A) or no treatment (Z), for example: 12:00 A differs from 12:00 P, 12:00 Z differs from 12:00 P and so on. 9:00 A and 9:00 Z differ from the intercept.*

Pupil diameter treated				**Confidence interval**	
Fixed effects	**Estimate difference (mm)**	**SEM**	**P value**	Lower bound	Upper Bound
Intercept*	5.74	0.280	0.00	5.20	6.29
12:00 P**	-1.42	0.218	0.00	-1.85	-1.00
15:00 P	-0.98	0.218	0.00	-1.40	-0.56
18:00 P	-0.74	0.218	0.00	-1.17	-0.32
21:00 P	0.30	0.218	0.18	-0.13	0.72
0:00 P	-0.24	0.218	0.27	-0.67	0.18
3:00 P	-1.56	0.218	0.00	-1.98	-1.13
6:00 P	-0.85	0.218	0.00	-1.27	-0.42
9:00 A***	0.54	0.218	0.01	0.12	0.97
12:00 A	0.17	0.218	0.45	-0.26	0.59
15:00 A	-0.13	0.218	0.56	-0.55	0.30
18:00 A	-0.01	0.218	0.98	-0.43	0.42
21:00 A	-0.18	0.220	0.40	-0.61	0.24
0:00 A	0.56	0.220	0.01	0.13	0.99
3:00 A	0.86	0.220	0.00	0.43	1.28
6:00 A	0.72	0.220	0.00	0.29	1.14
9:00 Z	0.35	0.252	0.17	-0.14	0.84
12:00 Z	-0.31	0.252	0.21	-0.80	0.18
15:00 Z	-0.83	0.252	0.00	-1.32	-0.34
18:00 Z	-0.06	0.252	0.81	-0.55	0.43

21:00 Z	-0.33	0.252	0.19	-0.82	0.16
0:00 Z	-1.03	0.252	0.00	-1.52	-0.54
3:00 Z	0.20	0.252	0.44	-0.29	0.68
6:00 Z	-0.37	0.252	0.14	-0.86	0.12

Table 14 - 95% confidence intervals of the horizontal pupil diameter of the untreated eye in the adjustment phase (Z), placebo phase (P) and treatment phase (A).

** The intercept values estimate the pupil diameter with lower and upper standard error at 9:00 when the other eye was treated with the placebo (P).*
*** Treatment with the placebo of the other eye (P) on different time points is to be seen as a difference in pupil diameter from the intercept.*
**** The treatment phase (A) and adjustment phase (Z) are to be seen as the pupil diameter difference at corresponding time points between placebo phase (P) to treatment phase (A) or adjustment phase (Z), for example: 12:00 A differs from 12:00 P, 12:00 Z differs from 12:00 P and so on. 9:00 A and 9:00 Z differ from the intercept.*

Pupil diameter untreated				**Confidence interval**	
Fixed effects	**Estimate difference (mm)**	**SEM**	**P value**	Lower bound	Upper Bound
Intercept*	5.74	0.306	0.00	5.15	6.34
12:00 P**	-1.41	0.217	0.00	-1.83	-0.99
15:00 P	-0.97	0.217	0.00	-1.39	-0.55
18:00 P	-0.77	0.217	0.00	-1.19	-0.35
21:00 P	0.31	0.217	0.15	-0.11	0.73
0:00 P	-0.24	0.217	0.26	-0.66	0.18
3:00 P	-1.56	0.217	0.00	-1.98	-1.14
6:00 P	-0.85	0.217	0.00	-1.27	-0.43
9:00 A***	1.27	0.217	0.00	0.85	1.69
12:00 A	0.85	0.217	0.00	0.43	1.27
15:00 A	0.45	0.217	0.04	0.03	0.87
18:00 A	0.64	0.217	0.00	0.22	1.06
21:00 A	0.54	0.218	0.01	0.12	0.96
0:00 A	1.15	0.218	0.00	0.73	1.57
3:00 A	1.38	0.218	0.00	0.96	1.80
6:00 A	1.18	0.218	0.00	0.76	1.61
9:00 Z	0.35	0.250	0.16	-0.14	0.83
12:00 Z	-0.33	0.250	0.19	-0.81	0.16
15:00 Z	-0.75	0.250	0.00	-1.24	-0.26
18:00 Z	-0.03	0.250	0.91	-0.51	0.46
21:00 Z	-0.30	0.250	0.24	-0.78	0.19
0:00 Z	-1.05	0.250	0.00	-1.53	-0.56
3:00 Z	0.20	0.250	0.44	-0.29	0.68
6:00 Z	-0.39	0.250	0.13	-0.87	0.10

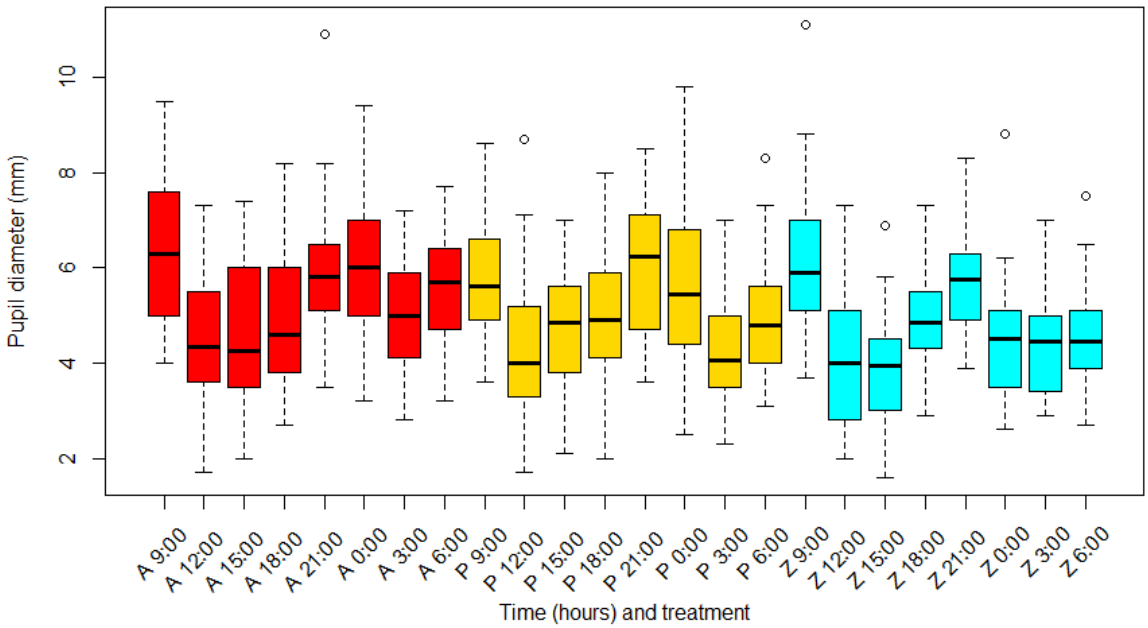

Figure 12 - Boxplot of the pupil diameter in the treated eye to the treatment and time points during treatment with dorzolamide (A), placebo (P) and no treatment (Z). All 5 days of measurement for the pupil diameter from the placebo and treatment phase were included in the calculation. For the adjustment phase only the last three days were included. Extreme values extending across the first and fourth quadrants are shown as dots.

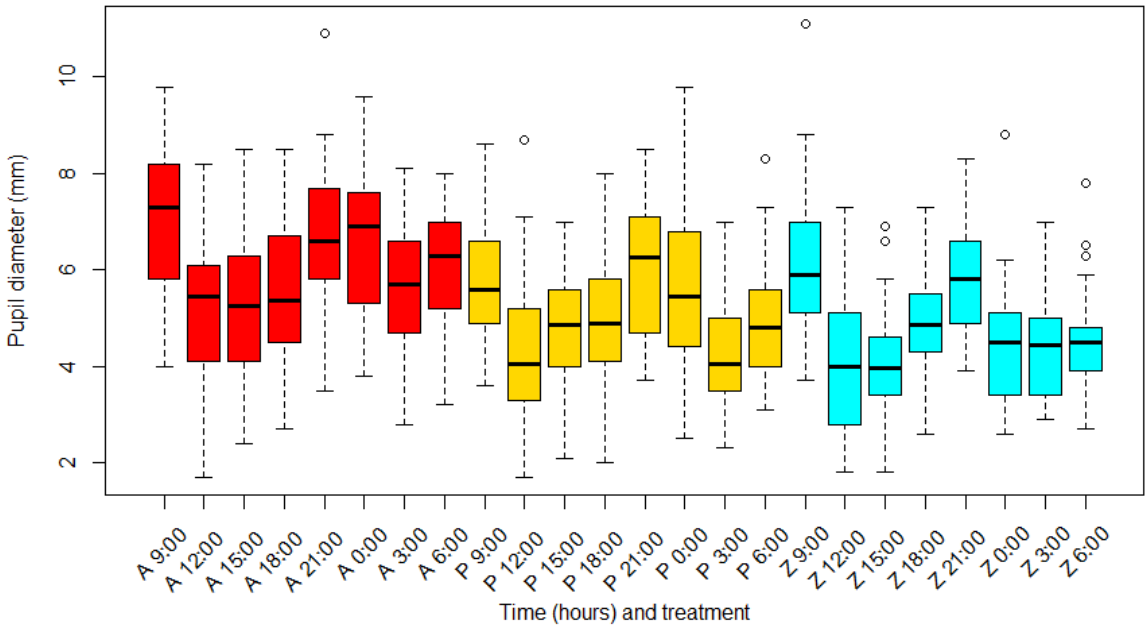

Figure 13 – Boxplot of the pupil diameter in the untreated eye to the treatment phases and time points during the treatment phase (A), placebo phase (P) and adjustment phase (Z). All 5 days of measurement for the pupil diameter from the placebo and treatment phase were included in the calculation. For the adjustment phase only the last three days were included. Extreme values extending across the first and fourth quadrants are shown as dots

Table 15 - 95% confidence intervals of the heart rate without treatment (Z), treatment with the placebo (P) and treatment with dorzolamide (A).

** The intercept values estimate the heart rate with lower and upper standard error at 9:00 for dorzolamide treatment (A).*
*** Treatment with dozolamide (A) on different time points is to be seen as a difference from the intercept.*
**** The effect of the placebo (P) and no treatment (Z) is to be seen as the heart rate difference at corresponding time points between the treatment with dorzolamide (A) and placebo (P) or no treatment (Z), for example: 12:00 Z differs from 12:00 A, 12:00 P differs from 12:00 A and so on. 9:00 P and 9:00 Z differ from the intercept.*

Heart rate				**Confidence interval**	
Fixed effects	**Estimate difference (BPM)**	**SEM**	**P value**	Lower bound	Upper Bound
Intercept*	160.16	4.226	0.00	151.96	168.36
12:00 A**	-2.56	3.666	0.49	-9.67	4.55
15:00 A	-2.88	3.666	0.43	-9.99	4.23
18:00 A	-1.36	3.666	0.71	-8.47	5.75
21:00 A	-6.97	3.685	0.06	-14.12	0.17
0:00 A	-3.55	3.685	0.34	-10.69	3.60
3:00 A	-6.73	3.685	0.07	-13.88	0.42
6:00 A	2.09	3.685	0.57	-5.06	9.23
9:00 Z***	-1.56	4.233	0.71	-9.77	6.65
12:00 Z	-11.73	4.233	0.01	-19.94	-3.52
15:00 Z	-13.28	4.233	0.00	-21.49	-5.07
18:00 Z	-14.00	4.233	0.00	-22.21	-5.79
21:00 Z	-7.99	4.249	0.06	-16.23	0.26
0:00 Z	-11.41	4.249	0.01	-19.66	-3.17
3:00 Z	1.90	4.249	0.65	-6.34	10.14
6:00 Z	-7.18	4.249	0.09	-15.42	1.06
9:00 P	2.48	3.666	0.50	-4.63	9.59
12:00 P	4.00	3.666	0.28	-3.11	11.11
15:00 P	-15.20	3.685	0.00	-22.34	-8.05
18:00 P	-18.56	3.666	0.00	-25.67	-11.45
21:00 P	-16.95	3.685	0.00	-24.09	-9.80
0:00 P	-10.05	3.685	0.01	-17.20	-2.91
3:00 P	7.45	3.685	0.04	0.30	14.60
6:00 P	3.39	3.723	0.36	-3.83	10.61

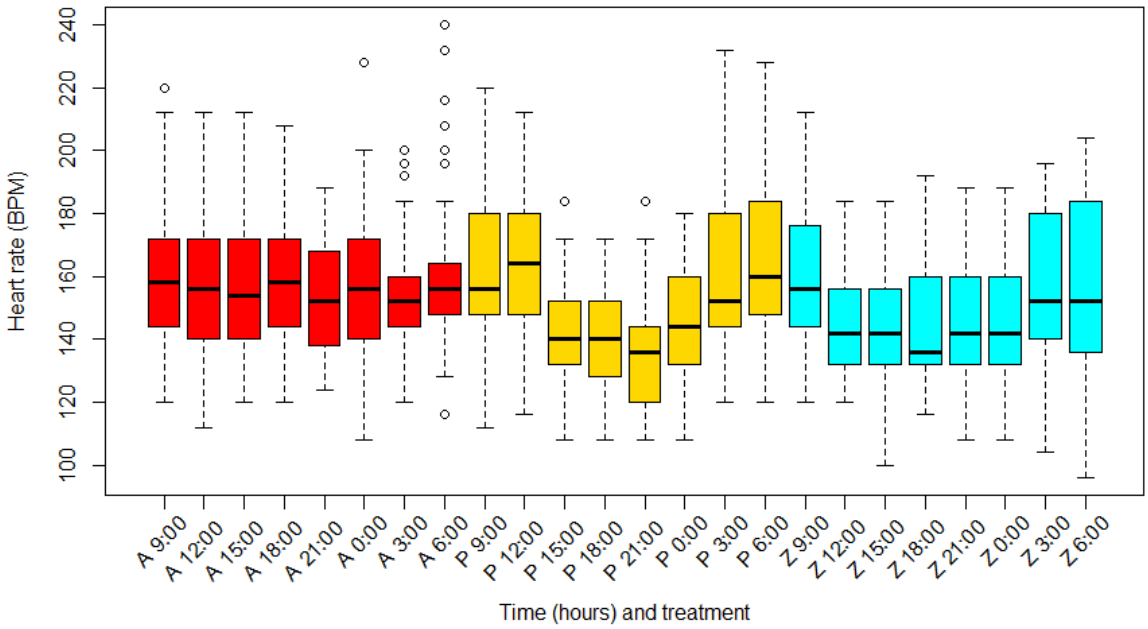

Figure 14 - Boxplot of the heart rate to the type of treatment and time points during treatment with dorzolamide (A), placebo (P) and no treatment (Z). All 5 days of measurement for the heart rate from the placebo and treatment phase were included in the calculation. For the adjustment phase only the last three days were included. Extreme values extending across the first and fourth quadrants are shown as dots.

Discussion

Adjustment IOP (mean ± SEM) in all 10 cats was established with a rebound tonometer at 16.43 ± 0.634 mm Hg. These findings were lower than previously reported values obtained with an applanation tonometer at 18.46 ± 2.99 mm Hg for 24 cats (4) and 19.7 ± 5.6 mm Hg for 41 cats (8), and with a rebound tonometer at 20.74 ± 0.48 mm Hg for 76 cats (9). Mean IOP greatly varied, as the cats with the lowest and highest mean IOP had 13.33 mm Hg and 17.24 mm Hg respectively in the adjustment phase (data not shown). When IOP measurements are summarised in days, the median is lower than the mean. This means there is more variance above the median than below (Tables 1, 2 and 3). In the literature, line graphs are often used to describe the results (4, 10). Also, boxplots are used to provide a summary of the distribution of a dataset (7). In this study, the same data is viewed in the form of boxplots (Figures 2, 4, 6) as well as line graphs (Figures 3, 5, 7) to provide the possibility for comparison of both data representations. Clear differences between median and mean are shown in Figures 2 and 3 on 21:00 to 6:00.

In addition, there could be considerable variance between IOP values in one set of three measurements (data not shown). Differences in IOP of 4-5 mm Hg were commonly found, while in literature 1-2 mm Hg differences were reported (9). The results of this study cannot explain the differences found in the literature.

IOP and variance decreased when the cats were measured a second time (“IOP2”) within 1-2 minutes after the first measurement (“IOP1”). This observation was made after the adjustment phase, and therefore the adjustment phase was only measured for IOP1. There is no current explanation as to why this effect occurred, although the research team speculated it could be arousal, increased activity level, or stress related.

A circadian IOP rhythm was found with peaks in the morning and a steady decline throughout the day, while another peak occurred around 21:00 (Tables 3-6 and Figures 2-5). Dorzolamide influenced the circadian rhythm, levelling the curves measured throughout a 24h-time period. The present study found a circadian rhythm with different peaks compared to previous studies (4, 10). An increase of more than 4-5 mm Hg in IOP during the night as reported by Del Sole et al. (10) was not found, possibly because in this study an automated light regime with an adjustment phase of 12h light-dark cycles was not used.
In some of the used models, gender was a fixed effect and the mean female IOP was higher than the male IOP (Tables 4, 5 and 6). In the literature, a difference in mean IOP values is only described between intact female cats and spayed female cats (3, 8). At present there is no explanation for the difference between male and female IOP. However, when the output of the LME model used to determine the difference between IOP of the treated and untreated eye was examined (Table 7 and 8), gender was not considered a relevant fixed effect. Based on the expert opinion of the Faculty of Veterinary Medicine statistician, it is common practice to drop a fixed effect from a linear mixed-effects model if the AIC value is only dropped by less than 2. Therefore the finding that gender is a fixed effect in some used LME models is probably coincidental. It is recommended that future studies should use more cats to lessen the chance of coincidences such as this.
The results in this study demonstrate statistically significant differences between the adjustment and placebo phase in three of the eight measured time points (Table 4). There is no explanation for this finding as the placebo should not have an IOP lowering effect (12). Like Dietrich et al. (4), a statistically significant difference between treatment with dorzolamide and the placebo was found on all measured time points. In this study a mean difference of 2.33 mm Hg between treatment with dorzolamide and the placebo was found for IOP1 (Table 5), while a mean difference of 3.21 mm Hg was found for IOP2 (Table 6). As mentioned earlier, there currently is no known explanation for the difference between IOP1 and IOP2. Pupil diameter of the untreated eye increased when the other eye was treated with timolol, the treated eye was not influenced by treatment (Tables 13 and 14 and Figures 12 and 13). Pupil diameter of both eyes decreased when light intensity increased. In this study, light intensity peaked at 12:00 (Table 12), which corresponds with the smallest pupil diameter measured during daytime. Heart rate did not change significantly over the course of the project (Table 15 and Figure 14).
There were no significant differences found between the treated eye and untreated eye in the placebo and treatment phases (Tables 7 and 8). The IOP ratio of the treated eye to the untreated eye was close to one in both the placebo and treatment phases (Figures 10 and 11). Possible explanations for these findings could be transfer of the medication with the probe, increased habituation of the cats during the project over time, or a systemic effect for topical treatment. However, transfer of the medication with the probe seems unlikely since the reduction of pupil diameter only occurred in the treated eye, and the amount of weeks did not influence the IOP (Tables 7 and 8 and Figures 8 and 9). It should be noted that none of these explanations are found in literature.
Topical administration of dorzolamide in healthy cats has been studied before (1, 4). In contrast to other studies, this study has an adjustment, placebo and treatment phase for all ten cats measured on eight different time points every 24 hours for 42 days excluding the days of rest. Since baseline values for each individual cat were collected, a control group was not necessary.

During the project one male cat appeared to have a systolic murmur, which upon cardiac and ultrasonographic examination led to a defect of the mitral valve. This cat received no additional medication for this defect till after this research. After this research the cat received treatment with atenolol, which in humans is known to cause a decrease in IOP (11). Over the course of the project, not the right type of medication was administered to the treated eye twice in two different cats. These data were removed from the set. Each day at 9:00 rooms of the kennel were cleaned and the cats were fed, which could have led to increased stress and arousal. 9:00 was the highest IOP measurement (Figures 2, 4 and 6), which could be explained by this increased activity level.
The present study found a significant IOP-lowering effect of topically applied dorzolamide 2% in healthy adult cats at all eight different time points throughout a 24h-period, as compared to a placebo. Future research into dorzolamide as a possible treatment for glaucoma in cats is warranted.

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