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Effects of 1% brinzolamide eye drops on the intraocular pressure, pupil diameter and heart rate in healthy cats.

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Abstract

Objective – To evaluate the effects of topical administration of 1% brinzolamide eye drops q8h on the circadian rhythm of the intraocular pressure in healthy domestic cats.
Animals – Ten adult, healthy domestic short-haired cats (five castrated males, five spayed females).
Procedure – This study was part of a larger study, in which the effects of four different topical medications were investigated. During the pre-treatment phase (day 1-6) no medication was administered. During the next placebo phase (day 7-12), artificial tears were administered to the eye selected for medication five times daily, at 7.30, 7.45, 15.30, 19.45 and 23.30 hours. On day 14-19, day 22-27, day 30-35 and day 38-43 the cats received topical medication. The protocols, which were rotated according to a Latin square, consisted of 5 days' administration of: A. dorzolamide 2% eye drops (at 7.30, 15.30 and 23.30 hours) and an artificial tear drop (at 7.45 and 19.45 hours); B. brinzolamide 1% eye drops (at 7.30, 15.30 and 23.30 hours) and an artificial tear drop (at 7.45 and 19.45 hours); C. dorzolamide 2% eye drops (at 7.30, 15.30 and 23.30 hours) and timolol 0.5% eye drops (at 7.45 and 19.45 hours); and D. brinzolamide 1% eye drops (at 7.30, 15.30 and 23.30 hours) and timolol 0.5% eye drops (at 7.45 and 19.45 hours). On each medication time point, an artificial teardrop was administered to the contralateral eye. Throughout the study intraocular pressure, pupil diameter, light intensity at eye level of both eyes and heart rate were measured q3h for 42 days. A linear mixed-effect model was fitted to the data. The time point 9:00h was selected as the reference for the purposes of comparisons.
Results – Overall mean IOP (+ S.E.) of the placebo was 13.6 ± 0.2 mmHg and overall mean IOP of brinzolamide was 11.7 ± 0.2 mmHg. The overall mean IOP reduced by 1.9 mmHg and 14 percent after administration of brinzolamide. The reduction of the IOP during the brinzolamide phase was significant at all time points, except at 00:00h. The pupil diameter of the treated eye during the brinzolamide phase was significantly larger at 4 time points compared to the treated eye of the placebo phase, despite the same light intensity conditions. There were no significant differences in heart rate between the placebo and brinzolamide phase.
Conclusion – Topical administration of brinzolamide 1% is effective in reducing the intraocular pressure in healthy, adult and domestic cats. However, more research is needed in cats with glaucoma.

Introduction

1.1 Glaucoma

Glaucoma is a neurodegenerative disease which consists of a group of different eye disorders, in which a high intraocular pressure is the most important risk factor for the development of glaucoma (1). It causes damage to the retina and optic nerve which leads to a loss of vision and eventually in irreversible blindness. Glaucoma can be classified based on the etiology into primary glaucoma and secondary glaucoma. In cats with primary glaucoma there are no other underlying or previous ocular disorders that causes glaucoma in contrast to secondary glaucoma. Most of the time, glaucoma in cats is secondary to an underlying disease (2). The causes of glaucoma in cats are very different, but the most important causes are neoplasia and chronic lympho-plasmatic uveitis (2,3). The clinical signs of glaucoma are not always explicitly present, but an important feature of glaucoma is an increased intraocular pressure.

1.2 Intraocular pressure

The intraocular pressure (IOP) in cats is an equilibrium between the aqueous humor outflow and the aqueous humor production. In a healthy cat eye, there is a balance between the outflow and production of aqueous humor. The aqueous is secreted by active and passive mechanisms and is mainly produced by the epithelium of the ciliary processes (4). Passive mechanisms are ultrafiltration and diffusion and active mechanisms are based on energy-dependent active secretion and produce against a gradient pressure (5). Several enzymatic processes are involved in the active secretion, in which carbonic anhydrase is one of the key enzymes. After secretion, most of the aqueous humor circulates from the posterior chamber, through the pupil, into the anterior chamber and drains through the irido-corneal angle into the trabecular meshwork which is located in the ciliary cleft of the drainage angle (4,5) (Figure 1). Then the humor drains to the angular aqueous plexus into the intra-scleral venous plexus and eventually into the general circulation. A small part of the aqueous humor leaves the eye via the uveoscleral route: through the iris and ciliary body to the suprachoroidal space and through the sclera (2). If an obstruction of the outflow of aqueous humor arises, for example due to infiltrates of neoplastic or inflammatory cells, and the active secretion is still functional, the IOP increases. Mean IOP (± S.E.) in healthy, adult cats using a rebound tonometer (TonoVet®) was reported to be 20.74 ± 0.5 mmHg (6). The severity and relevance of an elevated intraocular pressure are considered as follows (4).

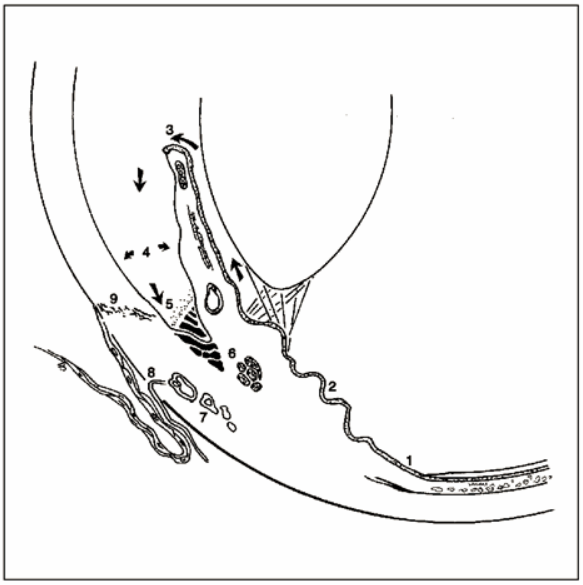

Figure 1: Aqueous humor secretion and outflow.
1: Pigment epithelium, 2: ciliary body, 3: pupil, 4: irido-corneal angle, 5: pectinate ligament, 6: drainage angle/ciliary cleft, 7: scleral venous plexus, 8: conjunctival vessel anastomosis, 9: limbus (Stades FC. Intraocular Pressure and Glaucoma. In: Ophthalmology for the Veterinary Practitioner. Second, revised and expanded. Hannover: Manson Publishing; 2007.p.157).

- 20-30 mmHg = moderately elevated
- > 20-30 mmHg = prophylactic therapy is warranted, depending on duration, clinical signs and results of additional ophthalmic examinations.
- > 40 mmHg = irreparable damage, depending on the duration

The IOP can be measured with tonometry and can be influenced by stress, age, activity, head position, eye movements, pressure on jugular vein during the measurement, some medications, reproductive status and the tonometer itself (2,7,8). However, some studies observed no influences of age (9). Beside this, the IOP in cats reportedly has a circadian rhythm during the day in which at night the values of the IOP are the highest (9). The IOP fluctuates about 4-5 mmHg during the day (2).

1.3 Glaucoma treatment

The goal of current glaucoma treatment is to decrease the IOP to protect the optic nerve. There are surgical and pharmacological treatments to lower the IOP. Surgical procedures are cyclodestruction, placing an implant to improve the drainage of the aqueous humor and a fistula method (4). However, pharmacological therapy is still important in the current treatment of glaucoma. Pharmacological therapy can reduce the IOP by lowering the production of the aqueous humor, by increasing the outflow of aqueous humor and/or by osmotically reducing intraocular volume. Routes of administration of pharmacological treatments are topical or systemic. The topical route is preferred, because of the systemic toxicity in cats by using the systematic route (10). Pilocarpine eye drops are the only registered topical medication for glaucoma in cats, but are contraindicated when glaucoma is secondary to uveitis, which is a common cause of feline glaucoma. Pilocarpine is a miotic drug and can increase uveitis (11,4). There are other pharmacological therapies for glaucoma, but most of them are registered for use in human medicine only. Topical medications include dorzolamide, brinzolamide and timolol. Brinzolamide is a carbonic anhydrase inhibitor and reduces the intraocular pressure by reducing the aqueous humor secretion (12). As mentioned before, carbonic anhydrase is one of the key enzymes in the active secretion of aqueous humor. Carbonic anhydrase is a catalyzer of the following reaction: $CO_2 + H_2O \leftrightarrow HCO_3^- + H^+$.
Bicarbonate (HCO_3^-) is important for the movement of sodium and water into the eye and therefore for the secretion of aqueous humor (10). Inhibition of carbonic anhydrase results in a reduction of the bicarbonate production and eventually in a decrease of the aqueous humor production. Brinzolamide has been evaluated in cat eyes. When brinzolamide 1% eye drops were applied twice a day, no significant reduction of the IOP was noted in normal cats (11). However, when applied three times a day, the IOP reduced significantly and the IOP fluctuations of the circadian rhythm also reduced in cats with primary glaucoma (13). Side effects may be topical irritation (brinzolamide less than dorzolamide), inappetence and hypersalvation.

1.4 Aim of the study

There is a lack of evidence-based veterinary medicine articles about the effects of intraocular pressure lowering drugs on the circadian rhythm of intraocular pressure in cats. Most of the articles use the intraocular pressure measured during day-time hours (14). Less is known about the effects of brinzolamide during night-time hours and about the effects of brinzolamide administered three times a day. The aim of the underlying study is to investigate the effects of the carbonic anhydrase inhibiting eye drops brinzolamide 1% on the circadian rhythm of intraocular pressure in healthy, adult cats.

Material and methods

2.1 Animals

The study population comprised twelve healthy, domestic short-haired cats of both sexes (six castrated males, six spayed females) with an average age of 1.87 (SD ± 0.04) years. All cats were part of a colony used for clinical teaching owned by the Department of Clinical Sciences, Faculty of Veterinary Medicine of Utrecht University. They were housed in two groups (males, females) under

standard, controlled environmental conditions. There was a light-dark regime, but this was not constant, because the light was turned on during the measurements at night. Each cat underwent a complete ophthalmic examination performed by a board-certified veterinary ophthalmologist (Dip. ECVO) to confirm ocular health before the study started. At every first day of the week before starting a new treatment the ophthalmic examination was repeated. The Animal Welfare Body Utrecht (IVD) provided permission for all the animal procedures.

2.2 Study design

This was a prospective, randomized, blind study. Examinations were performed by two teams of two persons each, taking shifts. Incidentally, two supervisors participated in the shifts. Throughout the study intraocular pressure, pupil diameter and light intensity at eye level of both eyes and heart rate were measured every three hours. The study was separated into six phases: a pre-treatment phase, a placebo phase, and four treatment phases with washout periods in between. The first five days were the pre-treatment phase and were used to get the observers and cats acclimated to the measurements. After these five days there was one day of rest. The next phase was the placebo phase and took also five days. After the placebo phase there was one day of rest and one day of measuring all parameters. Then, there were four treatment periods. Cats were randomly assigned to one of four groups using the Random Team Generator and the protocols were rotated according to a Latin square. Each treatment period consisted of five days and was followed by a washout period which consisted of two days of rest and one day of measuring all parameters, except after the last treatment period (Figure 2).

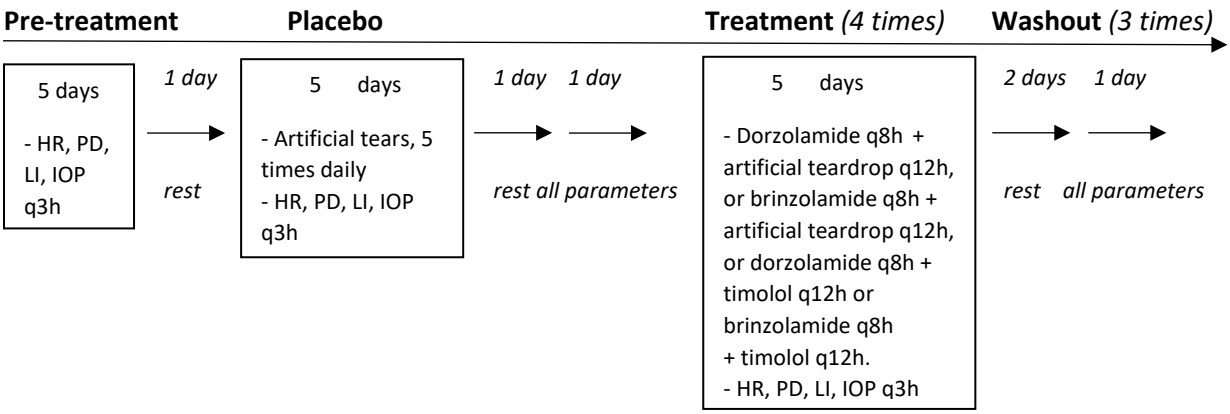

Figure 2. Schematic representation of study design.
The heart rate (HR), pupil diameter (PD), light intensity at eye level of both eyes (LI) and intraocular pressure (IOP) were measured every 3 hours in 10 healthy, adult cats during 42 days. Measurements were performed at 9:00, 12:00, 15:00, 18:00, 21:00, 00:00, 3:00 and 6:00 hours. In the placebo phase, artificial tears were administered to the eye selected for medication 5 times daily at 7.30, 7.45, 15.30, 19.45 and 23.30 hours. Medication was administered every 8 and every 12 hours to one randomly selected eye and an artificial teardrop was administered to the contralateral control eye. During the days of rest and the days of the measurements of all parameters, no medication was administered.

2.3 Drug administration

The eye to be medicated every 8 and 12 hours was randomly assigned using the flip a coin method. During the pre-treatment phase (day 1-6) no medication was administered. During the placebo phase (day 7-12), artificial tears (Lacriforte®, AST Farma B.V., Oudewater, The Netherlands) were administered to the eye selected for medication five times daily, at 7.30, 7.45, 15.30, 19.45 and 23.30 hours, while the other eye did not receive any medication in order to acclimate the cats to eye drops.

Medication administered during the treatment periods (day 14-19, day 22-27, day 30-35, day 38-43) included dorzolamide 2% eye drops (Cetrafarm B.V., Etten-Leur, The Netherlands), brinzolamide 1% eye drops (Azopt®, Alcon, Camberley, UK), timolol 0.5% eye drops (Sandoz, Almere, The Netherlands) and artificial tear drops (Lacriforte®, AST Farma B.V., Oudewater, The Netherlands). The four groups of cats were subjected to four different medication protocols according to a Latin square design. The protocols consisted of 5 days' administration of: A. dorzolamide 2% eye drops (at 7.30, 15.30 and 23.30 hours) and an artificial tear drop (at 7.45 and 19.45 hours); B. brinzolamide 1% eye drops (at 7.30, 15.30 and 23.30 hours) and an artificial tear drop (at 7.45 and 19.45 hours); C. dorzolamide 2% eye drops (at 7.30, 15.30 and 23.30 hours) and timolol 0.5% eye drops (at 7.45 and 19.45 hours); and D. brinzolamide 1% eye drops (at 7.30, 15.30 and 23.30 hours) and timolol 0.5% eye drops (at 7.45 and 19.45 hours). On each medication time point, an artificial teardrop was administered to the contralateral eye. For each cat, an individual medication vial was used and after administration of the medication, ocular irritation was monitored using a scale from 0 till 2 based on the pinching of the eyes (0=no pinching, 2= pinching >30 sec). During the washout periods of three days, no medication was administered. The examiners were blinded to the medication.

2.4 Measured parameters

Eight times a day, at 3-hours intervals, the intraocular pressure, pupil diameter, light intensity at eye level of both eyes and heart rate were measured. These measurement moments were at: 0:00h, 3:00h, 6:00h, 9:00h, 12:00h, 15:00h, 18.00h and 21.00h. One day was measured from 9.00 – 6.00 h and the cats were examined in the same order during the study. To measure the intraocular pressure, a rebound tonometer (TonoVet®, ICare, Vantaa, Finland) was used according to instructions of the manufacturer. The measurements were always performed in the right eye first and a series of three valid measurements was obtained in both eyes. Starting in the placebo period, a second series of three measurements was obtained, immediately after the first series and in the same order (right eye first). The horizontal pupil diameter was measured using digital calipers (kwb Germany GmbH, Stuhr, Germany) positioned a few millimeters anterior to the cornea. The luminance conditions under which the pupil diameter was measured, were determined at the level of the examined eyes using a luxmeter (Mastech Luxmeter MS6610, MastechDigital, Pittsburg, Pennsylvania, USA). The heart rate was determined by thoracic auscultation using a 3M™ Classic II S.E. Littmann stethoscope. During the measurements the head of the cats was maintained in a normal position, the chin being supported by hand. Care was taken not to apply pressure to the neck.

2.5 Statistical analysis

Time of the measurements were set as follows: 9:00h (time 1), 12:00h (time 2), 15:00h (time 3), 18.00h (time 4), 21.00h (time 5), 0:00h (time 6), 3:00h (time 7) and 6:00h (time 8). The IOP was measured in two sets of three measurements in both eyes. In the statistical analysis the mean of the first or second set of three measurements of the IOP for that eye was used. A linear mixed-effect model was used with a fixed effect for treatment, day, time, treatment by day interactions, treatment by time interactions, heart rate, right or left eye treated, treated-untreated eye, light intensity, gender or pupil diameter. A random effect was fitted for the repeated measurements at each time point for each individual cat, to take into account that each cat has individual differences. The acquisition model was determined based on the lowest Akaike Information Criterion (AIC) value with an ANOVA. To check the acquisition model, a visual inspection of the residues was done. The time point 9:00h was selected as the reference for the purposes of comparisons. The estimated coefficients indicate the average difference between the fixed effects. RStudio version 0.99.903 was used for the computations. The differences were considered statistically significant if $P < 0.05$.

Results

3.1 Intraocular pressure

3.1.1 Pre-treatment and placebo

To compare the placebo phase with the pre-treatment phase, only the measurements of set 1 of the IOP could be used, because the second set of measurements of the IOP was not obtained in the pre-treatment phase. Figure 3 shows the mean IOP of each day of the pre-treatment phase and the placebo phase. The figure shows a lot of variation of the IOP on day 1 and 2 of the pre-treatment phase. Beside this, in the first two days of the pre-treatment phase many measurements were missing and the time between the measurement moments was too close, because the observers and the cats needed to get accustomed to the procedures. For these reasons, day 1 and day 2 were deleted from the dataset of the pre-treatment phase. Only day 3,4 and 5 of the pre-treatment and placebo phase were compared.

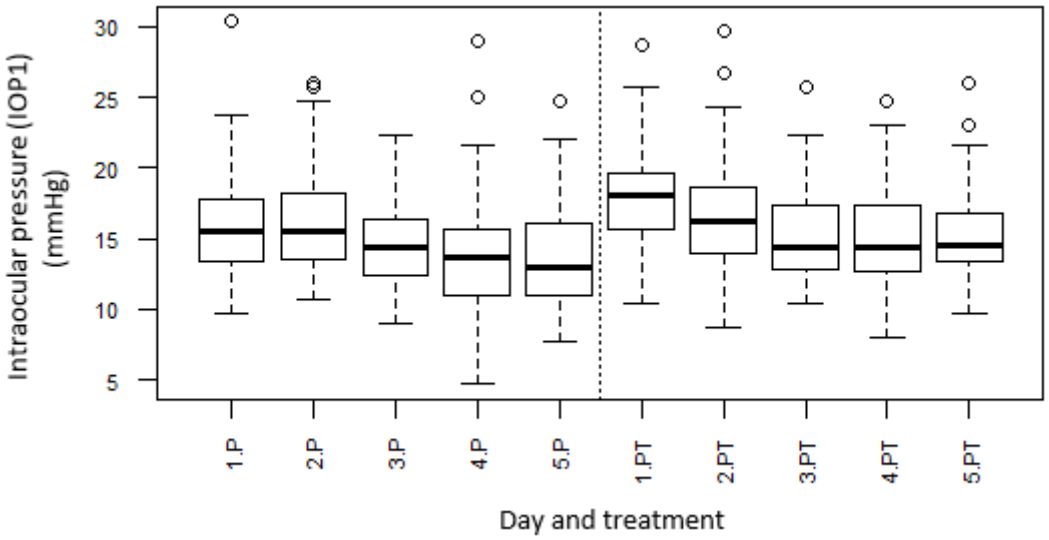

Figure 3. *Boxplots of the mean intraocular pressure at each day (day 1 till day 5) during the placebo phase (P) at the left side and during the pre-treatment phase (PT) at the right side of the figure in ten healthy, adult cats. During day 1 and day 2 of the pre-treatment phase (PT) there was a lot of variation of the IOP. During the placebo phase (P) the IOP of day 1 and day 2 was significantly higher than of day 3,4 and 5.*

Circadian rhythm pre-treatment and placebo

After analysis of the linear-mixed effect model (fitted values of the model: time, time-treatment interaction, gender and pupil diameter) a significantly higher IOP at different time points during the pre-treatment phase was observed compared to the placebo phase. The significant differences between the placebo and pre-treatment are marked with a star at the X-line (Figure 4). In Table 1, the mean differences in IOP along with the confidence intervals and P values are shown. Day, day by treatment interaction, left or right eye treated, heart rate, pupil diameter or gender did not influence the IOP.

Figure 4 also showed a peak value of the IOP during the placebo phase at 9:00 and 12:00 hours and the lowest value at 18:00h. Mean difference (+ confidence interval) at 18:00h was -2.3 mmHg (-3.6 to -0.9 mmHg) compared to 9:00h ($P < 0.01$) (Table 1). Significant differences within the placebo phase are marked with a tetragon in the figure. During the pre-treatment phase, the peak IOP occurred also at 9:00h and the lowest value also at 18:00h. Significant differences within the pre-treatment phase are marked with a triangle in the figure.

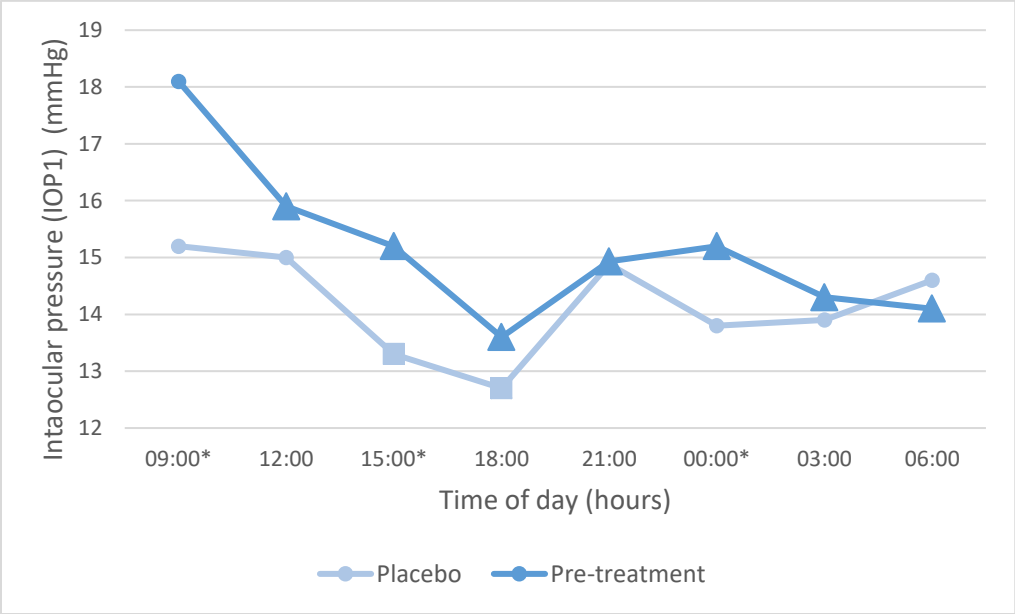

Figure 4. *Mean intraocular pressure of measurements of set 1 in the pre-treatment phase, as compared to topical administration of an artificial teardrop five times daily (placebo) in 10 healthy, adult cats at different times of the day.*

** Significant difference in IOP of placebo and pre-treatment at this time.*
Δ IOP at this time point differs significantly from IOP at 9:00h during pre-treatment phase.
□ IOP at this time point differs significantly from IOP at 9:00h during placebo phase.

Treated versus untreated eye

In the linear-mixed effect model with the following fixed effects: time, day, time-day interaction and treated-untreated eye, the IOP of the treated eye compared to the untreated contralateral control eye showed no significant difference during the pre-treatment phase and during the placebo phase ($P > 0.05$).

Table 1. Results of the linear mixed-effect model: placebo versus pre-treatment. *This model compared mean IOP (mmHg) of the measurements of set 1 in 10 healthy, adult cats, during the pre-treatment phase in which no medication was administered as compared to topical administration of an artificial tear drop five times daily (placebo). Estimate coefficients indicate the average difference between the intercept and the other variables. The 95% confidence intervals and P values are also shown.*

Variable	Estimate	P-value	95% confidence	interval
Time				
Placebo				
Intercept[1]	12.51	0.0000	10.52	14.50
Time 2: 12.00h	0.02	0.9804	-1.32	1.35
Time 3: 15:00h	-1.60	0.0226*	-2.96	-0.25
Time 4: 18:00h	-2.28	0.0012*	-3.62	-0.93
Time 5: 21:00h	-0.34	0.6160	-1.67	0.98
Time 6: 00:00h	-1.20	0.0838	-2.54	0.14
Time 7: 03:00h	-0.91	0.2015	-2.28	0.46
Time 8: 06:00h	-0.39	0.5699	-1.72	0.94
Time * treatment interaction				
Pre-treatment				
Time 1: 09:00h	2.72	0.0001*	1.39	4.05
Time 2: 12.00h	1.13	0.1030	-0.20	2.47
Time 3: 15:00h	2.07	0.0028*	0.74	3.39
Time 4: 18:00h	0.78	0.2552	-0.54	2.11
Time 5: 21:00h	-0.03	0.9599	-1.36	1.29
Time 6: 00:00h	1.46	0.0344*	0.13	2.78
Time 7: 03:00h	0.36	0.5968	-0.96	1.69
Time 8: 06:00h	-0.41	0.5556	-1.73	0.92
Gender female	2.25	0.0331*	0.27	4.23
Pupil diameter	0.29	0.0271*	0.04	0.53

[1] Mean IOP (mmHg) placebo time 1 (9:00 h)

* Indicates P < 0.05

3.1.2 Placebo and brinzolamide

To compare the placebo period with the brinzolamide period only the last 3 days were used for the analysis, because the IOP in the placebo phase seemed to stabilize only after two days (Figure 3). The IOP of the last 3 days was significantly lower than of day 1 and 2 in the placebo phase ($P < 0.01$). Estimate mean IOP (+confidence interval) of day 1 was 15.7 mmHg (C.I. 14.3 to 17.1 mmHg), mean difference IOP (+ C.I.) of day 3 was -1.1 mmHg (C.I. -1.7 to -0.4 mmHg), mean difference IOP (+ C.I.) of day 4 was -1.8 mmHg (C.I. -2.5 to -1.1 mmHg) and mean difference IOP (+ C.I.) of day 5 was -1.8 mmHg (C.I. -2.5 to -1.1 mmHg) ($P < 0.01$).
Overall mean IOP of set 1 (IOP1) (+ S.E.) of the placebo phase was 14.2 ± 0.6 mmHg and overall mean IOP of set 2 (IOP2) of the placebo phase was 13.6 ± 0.2 mmHg. Overall mean IOP1 of the brinzolamide phase was 12.5 ± 0.4 mmHg and the overall mean IOP2 of the brinzolamide phase was 11.7 ± 0.2 mmHg. The differences between set 1 and set 2 of the IOP were significant in both the placebo ($P = 0.02$) and brinzolamide phase ($P < 0.01$). The IOP of set 1 compared to the IOP of set 2 showed much more variation and was significantly higher, both in the placebo and the brinzolamide phase. Therefore, the second set of measurements of the IOP (IOP2) was used for the analysis.

Circadian rhythm placebo and brinzolamide

After analysis of the linear-mixed effect model (fitted values of the model: time and time-treatment interaction) a significant reduction of the IOP during the brinzolamide phase compared to the placebo phase was observed at all times, except at 00:00h (Figure 5). The significant differences between the IOP of the placebo phase and the IOP of the brinzolamide phase are marked with a star at the X-line in the figure. In Table 2, the mean differences in IOP along with the confidence intervals and P values are shown. Overall mean IOP (+ S.E.) of the placebo was 13.6 ± 0.2 mmHg and overall mean IOP of brinzolamide was 11.7 ± 0.2 mmHg. The IOP reduced by 14 percent during the brinzolamide phase. Day, day by treatment interaction, left or right eye treated, heart rate, pupil diameter or gender did not influenced the IOP.

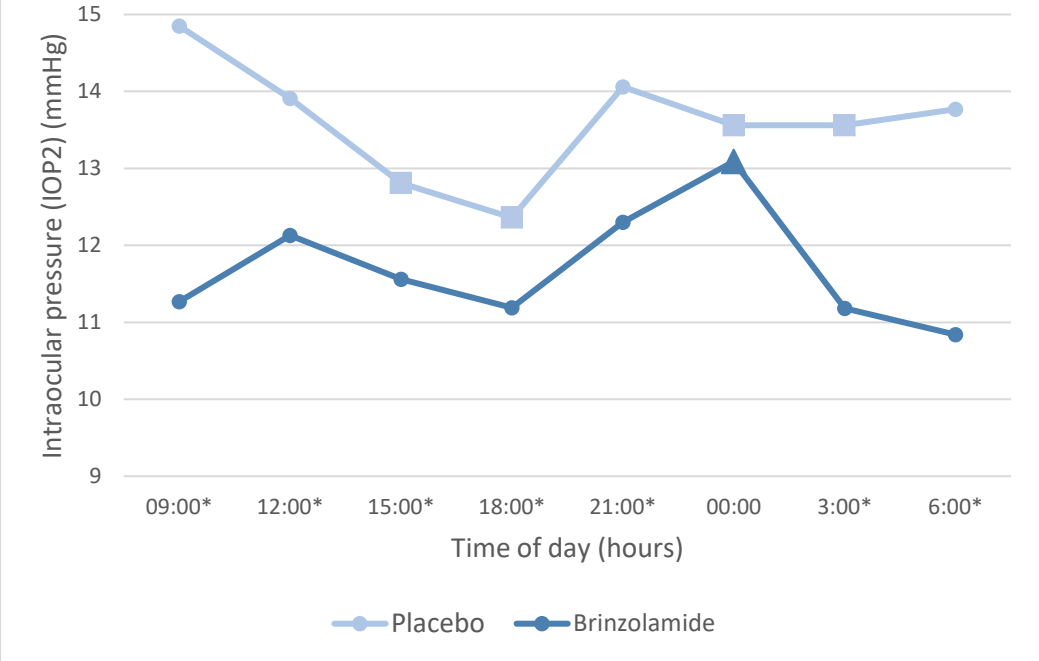

Figure 5. *Mean intraocular pressure of set 2 after topical administration of an artificial teardrop five times daily (placebo) as compared to topical administration of a brinzolamide 1% teardrop q8h and an artificial teardrop q12h in 10 healthy, adult cats at different times of the day.*

** Significant difference in IOP of placebo and brinzolamide at this time.*
Δ IOP at this time point differs significantly from IOP at 9:00h during brinzolamide phase.
□ IOP at this time point differs significantly from IOP at 9:00h during placebo phase.

Figure 5 shows a peak value of the IOP during the brinzolamide phase at 0:00h and the lowest value at 6:00h. Compared to 9:00h, mean difference with IOP at 0:00h was 1.8 mmHg (C.I. 0.7 – 3.0 mmHg) and mean difference with IOP at 6:00h was -0.4 mmHg (C.I. -1.6 – 0.7 mmHg) ($P < 0.01$) (Table 2). In

the placebo phase, the time points 15:00, 18:00, 0:00 and 3:00 hours were significantly different from 9.00h. These significant differences within the placebo phase are marked with a tetragon in the figure. During the brinzolamide phase there was only one significant fluctuation at 0:00 hours compared to 9:00h, which is marked with a triangle in the figure. The maximum fluctuation of the IOP in the placebo phase was 2.5 mmHg and during the brinzolamide phase it was 1.8 mmHg.

Treated versus untreated eye

The IOP of the treated eye compared to the contralateral untreated eye showed no significant difference during the placebo phase in the linear-mixed effect model with the following fixed effects: time, day, time-day interaction and treated-untreated eye. Mean IOP (± S.E.) treated eye was 13.6 ± 0.5 mmHg and mean IOP untreated eye was 13.5 ± 0.2 mmHg. During the brinzolamide phase there was also no significant difference between the treated and untreated eye. Mean IOP (± S.E.) treated eye was 11.7 ± 0.4 mmHg and mean IOP untreated eye was 11.8 ± 0.2 mmHg.

Table 2. Results of the linear mixed-effect model: placebo versus brinzolamide treatment. *This model compared the mean IOP (mmHg) of the measurements of set 2 in 10 healthy, adult cats after topical administration of brinzolamide 1% eye drops q8h and an artificial tear drop q12h, as compared to topical administration of an artificial tear drop five times daily (placebo). Estimate coefficients indicate the average difference between the intercept and the other variables. The 95% confidence intervals and P values are also shown.*

Variable	Estimate	P-value	95% confidence	interval
Time				
Placebo				
Intercept[1]	14.85	0.0000	13.80	15.91
Time 2: 12.00h	-0.94	0.1060	-2.07	0.18
Time 3: 15:00h	-2.04	0.0005*	-3.17	-0.92
Time 4: 18:00h	-2.49	0.0000*	-3.61	-1.36
Time 5: 21:00h	-0.79	0.1767	-1.91	0.34
Time 6: 00:00h	-1.29	0.0275*	-2.41	-0.16
Time 7: 03:00h	-1.29	0.0275*	-2.41	-0.16
Time 8: 06:00h	-1.08	0.0652	-2.20	0.05
Time * treatment interaction				
Brinzolamide				
Time 1: 09:00h	-3.58	0.0000*	-4.70	-2.45
Time 2: 12.00h	-1.78	0.0024*	-2.90	-0.65
Time 3: 15:00h	-1.25	0.0330*	-2.37	-0.12
Time 4: 18:00h	-1.17	0.0461*	-2.29	-0.04
Time 5: 21:00h	-1.76	0.0027*	-2.88	-0.63
Time 6: 00:00h	-0.47	0.4239	-1.59	0.66
Time 7: 03:00h	-2.38	0.0001*	-3.50	-1.25
Time 8: 06:00h	-2.93	0.0000*	-4.06	-1.81

[1] Mean IOP (mmHg) placebo time 1 (9:00h)

* Indicates $P < 0.05$

3.2 Pupil diameter and light intensity

3.2.1 Placebo and brinzolamide

A linear-mixed effect model with the fixed effects: time, time-treatment interaction and light intensity, showed a larger pupil diameter of the treated eye during the brinzolamide phase compared to the placebo phase. This was significant at four times of the day, which are marked with a star at the X-line in Figure 6 (Table 3). Overall mean (+S.E.) pupil diameter in the placebo period was 4.8 ± 0.3 mm and overall mean pupil diameter in the brinzolamide period was 5.3 ± 0.1 mm. Day was also a fixed effect, but was not taken into account in the analysis. Day by treatment, left or right eye treated, gender, IOP, or heart rate did not influenced the pupil diameter. Light intensity did influence the pupil diameter (see subheading Light Intensity at p. 14).

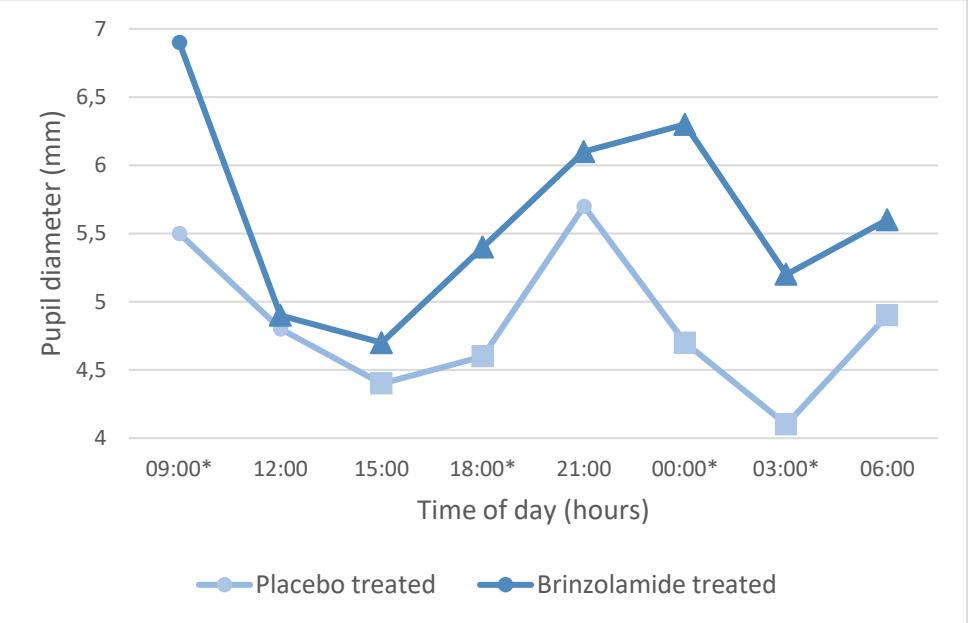

Figure 6. *Mean pupil diameter (PD) of the treated eye after topical administration of an artificial teardrop five times daily (placebo) as compared to topical administration of a brinzolamide 1% teardrop q8h and an artificial teardrop q12h in 10 healthy, adult cats at different times of the day.*

** Significant difference PD of placebo and brinzolamide at this time.*
Δ PD at this time point differs significantly from PD at 9:00h during brinzolamide phase.
□ PD at this time point differs significantly from PD at 9:00h during placebo phase.

Treated and untreated eyes

The linear-mixed effect model with the following fixed effects: time, day, time-day interaction and treated-untreated eye showed no significant differences between the treated and untreated contralateral eyes for the pupil diameter during the placebo phase and brinzolamide phase (P > 0.05).

Table 3. Results of the linear mixed-effect model: placebo versus brinzolamide treatment. *This model compared the pupil diameter (mm) in 10 healthy, adult cats after topical administration of brinzolamide 1% eye drops q8h and an artificial tear drop q12h, as compared to topical administration of an artificial tear drop five times daily (placebo). Estimate coefficients indicate the average difference between the intercept and the other variables. The 95% confidence intervals and P values are also shown.*

Variable	Estimate	P-value	95% confidence	interval
Time				
Placebo				
Intercept[1]	5.90	0.0000	5.21	6.59
Time 2: 12.00h	-0.48	0.0620*	-0.97	0.02
Time 3: 15:00h	-1.15	0.0000*	-1.63	-0.67
Time 4: 18:00h	-0.98	0.0001*	-1.46	-0.50
Time 5: 21:00h	0.13	0.5989	-0.35	0.61
Time 6: 00:00h	-0.88	0.0004*	-1.36	-0.40

Time 7: 03:00h	-1.44	0.0000*	-1.91	-0.96
Time 8: 06:00h	-0.65	0.0093*	-1.13	-0.17
Time * treatment interaction				
Brinzolamide				
Time 1: 09:00h	1.04	0.0000*	0.56	1.51
Time 2: 12.00h	-0.26	0.2940	-0.74	0.22
Time 3: 15:00h	0.13	0.5878	-0.34	0.61
Time 4: 18:00h	0.54	0.0301*	0.06	1.02
Time 5: 21:00h	0.13	0.6159	-0.36	0.61
Time 6: 00:00h	1.39	0.0000*	0.91	1.86
Time 7: 03:00h	0.88	0.0004*	0.40	1.35
Time 8: 06:00h	0.48	0.0546	0.00	0.95
Light intensity	0.00	0.0029*	0.00	0.00

[1] Mean pupil diameter (mm) placebo time 1 (9:00h)

* Indicates P < 0.05

Light intensity

A linear-mixed effect model with the fixed effects: time and time-treatment interaction, showed only at time point 21:00h a significant difference in light intensity between the placebo and brinzolamide period (P = 0.03) (Table 4). Overall mean (+S.E.) light intensity in the placebo period was 234 ± 8 lux and overall mean light intensity in the brinzolamide period was 252 ± 9 lux. During both the placebo and brinzolamide period, the light intensity at 12:00h differs significantly from the light intensity at 9:00h (P < 0.01) (Figure 7). Also at 12:00h, the peak value of the light intensity occurred in both the placebo and brinzolamide period. During the placebo period the light intensity at 18:00h and 21:00h was also significantly different from the light intensity at 9:00h (P<0.03), which is marked with a tetragon in the figure. Day was also a fixed effect, but was not taken into account in the analysis.

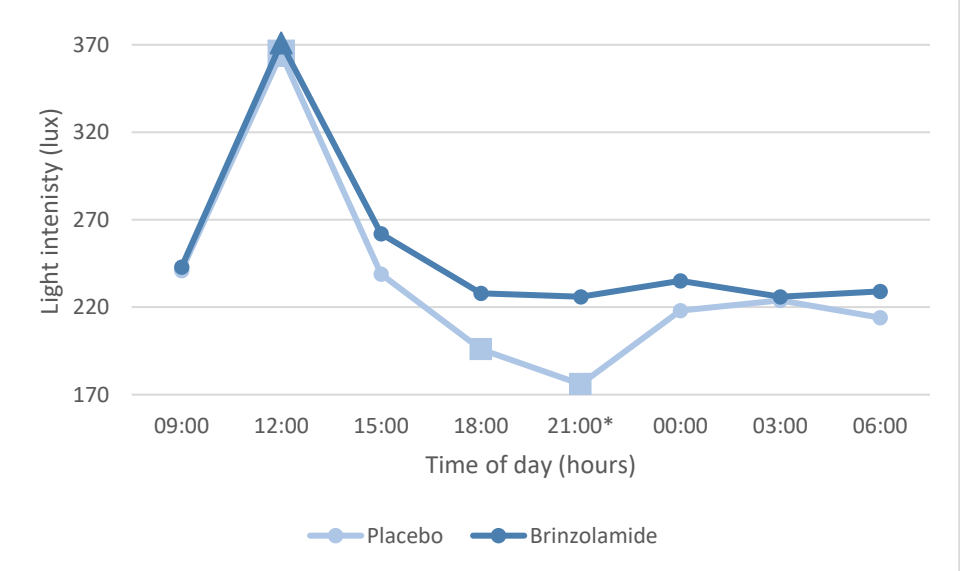

Figure 7. *Mean light intensity (LI) of the treated eye after topical administration of an artificial teardrop five times daily (placebo) as compared to topical administration of a brinzolamide 1% teardrop q8h and an artificial teardrop q12h in 10 healthy cats at different times of the day.*

** Significant difference in LI of placebo and brinzolamide at this time.*
Δ LI at this time point differs significantly from LI at 9:00h during brinzolamide phase.
□ LI at this time point differs significantly from LI at 9:00h during placebo phase.

Table 4. Results of the linear mixed-effect model: placebo versus brinzolamide treatment. *This model compared the light intensity (lux) in 10 healthy, adult cats after topical administration of brinzolamide 1% eye drops q8h and an artificial tear drop q12h, as compared to topical administration of an artificial tear drop five times daily (placebo). Estimate coefficients indicate the average difference between the intercept and the other variables. The 95% confidence intervals and P values are also shown.*

Variable	Estimate	P-value	95% confidence	interval
Time				
Placebo				
Intercept[1]	241.37	0.0000	210.55	272.18
Time 2: 12.00h	123.77	0.0000*	82.36	165.17
Time 3: 15:00h	-2.77	0.8973	-44.17	38.64
Time 4: 18:00h	-45.73	0.0334*	-87.14	-4.33
Time 5: 21:00h	-64.90	0.0026*	-106.30	-23.50
Time 6: 00:00h	-23.13	0.2809	-64.54	18.27
Time 7: 03:00h	-17.50	0.4146	-58.90	23.90
Time 8: 06:00h	-27.13	0.2061	-68.54	14.27
Time * treatment interaction				
Brinzolamide				
Time 1: 09:00h	1.43	0.9467	-39.97	42.84
Time 2: 12.00h	6.27	0.7701	-35.14	47.67
Time 3: 15:00h	23.23	0.2788	-18.17	64.64
Time 4: 18:00h	32.60	0.1289	-8.80	74.00
Time 5: 21:00h	49.34	0.0229*	7.58	91.10
Time 6: 00:00h	16.77	0.4344	-24.64	58.17
Time 7: 03:00h	2.33	0.9133	-39.07	43.74
Time 8: 06:00h	14.50	0.4990	-26.90	55.90

[1] Mean light intensity (lux) placebo time 1 (9:00h)

* Indicates $P < 0.05$

3.3 Heart rate

Mean overall heart rate (± S.E.) during the placebo period was 151 ± 4 bpm. During the brinzolamide period, mean overall heart rate (± S.E.) was 153 ± 2 bpm. No significant differences of the overall heart rate between placebo and brinzolamide were found (P = 0.24). A linear-mixed effect model with the fixed effects: time, day and time-treatment interaction was used. Gender, day-treatment interaction, left or right eye treated, IOP and pupil diameter did not influenced the heart rate. There were, however, significant differences in heart rate depending on the time of measuring. A significant difference in heart rate between the placebo and brinzolamide phase at 15:00, 18:00, 21:00 and 03:00 hours was observed (P < 0.05) (Table 4). However, there was much variation of the heart rate between the time points and within the time points during the placebo and brinzolamide phase (Figure 7). During the placebo phase, the heart rate seemed to follow a circadian rhythm, but during the brinzolamide phase the circadian rhythm was much more difficult to recognize.

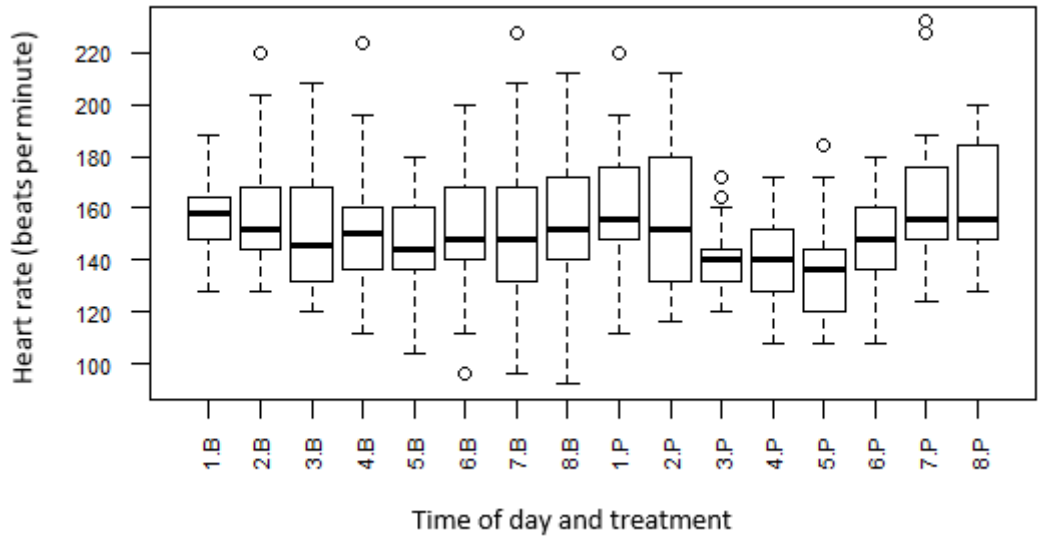

Figure 7. *Boxplots of the heart rate at the individual time points of the day (time 1 till 8) during the brinzolamide phase (B) at the left side and during the placebo phase (P) at the right side of the figure in ten healthy, adult cats.*

Table 4. Results of the linear mixed-effect model: placebo versus brinzolamide treatment. *This model compared the heart rate (bpm) of 10 healthy, adult cats after topical administration of brinzolamide 1% eye drops q8h and an artificial tear drop q12h, as compared to topical administration of an artificial tear drop five times daily (placebo). Estimate coefficients indicate the average difference between the intercept and the other variables. The 95% confidence intervals and P values are also shown.*

Variable	Estimate	P-value	95% confidence	interval
Time				
Placebo				
Intercept[1]	161.59	0.0000	151.23	171.95
Time 2: 12.00h	-4.80	0.2913	-13.56	3.96
Time 3: 15:00h	-21.81	0.0000*	-30.64	-12.97
Time 4: 18:00h	-22.80	0.0000*	-31.56	-14.04
Time 5: 21:00h	-25.73	0.0000*	-34.49	16.98
Time 6: 00:00h	-13.73	0.0026*	-22.49	-4.98
Time 7: 03:00h	-0.13	0.9766	-8.89	8.62
Time 8: 06:00h	2.41	0.5990	-6.42	11.25

Day				
Day 4	-2.68	0.1752	-6.50	1.13
Day 5	3.11	0.1147	-0.68	6.91
Time * treatment interaction				
Brinzolamide				
Time 1: 09:00h	-4.93	0.2781	-13.69	3.82
Time 2: 12.00h	0.80	0.8603	-7.96	9.56
Time 3: 15:00h	12.08	0.0087*	3.24	20.91
Time 4: 18:00h	13.87	0.0024*	5.11	22.62
Time 5: 21:00h	9.80	0.0331*	0.96	18.63
Time 6: 00:00h	2.80	0.5380	-5.96	11.56
Time 7: 03:00h	-9.73	0.0327*	-18.49	-0.98
Time 8: 06:00h	-8.34	0.0693	-17.18	0.49

[1] Mean heart rate (bpm) placebo time 1 (9:00h)

* Indicates $P < 0.05$

3.4 Ophthalmic examination and scores of ocular irritation

During the study, no ophthalmic abnormalities were found that were relevant to the research project. Cats did not develop any blepharospasm or ocular discharge and there were no signs of corneal edema or conjunctival swelling or hyperemia throughout the study.

There were some scores of pinching of the eyes for less than 30 seconds after the administration of the brinzolamide eye drops (Table 5). There were no cats who pinched for more than 30 seconds.

Table 5. Results of the scores of ocular irritation after the administration of brinzolamide 1% eye drops q8h and an artificial tear drop q12h, 5 times daily in 10 healthy, adult cats. *The scores are on a scale of 1 till 5, 1/5 means there was pinching of the eyes at one medication time point, 2/5 means there was pinching of the eyes during two medication time points etc. During the measurements of cats 1 till 3 , the scores of 3 medication moments were missing. The pinching was for less than 30 seconds. The question mark means the scores were missing.*

	Cat 1	**Cat 2**	**Cat 3**	**Cat4**	**Cat 5**	**Cat 6**	**Cat 7**	**Cat 8**	**Cat 9**	**Cat 10**
Day 1	0/2	?	?	1/5	2/5	0/5	1/5	1/5	2/5	2/5
Day 2	1/2	0/2	0/2	0/5	0/5	1/5	0/5	1/5	1/5	3/5
Day 3	0/2	0/2	0/2	1/5	1/5	1/5	0/5	3/5	2/5	2/5
Day 4	0/2	0/2	0/2	0/5	2/5	1/5	1/5	2/5	1/5	1/5
Day 5	1/2	0/2	2/2	1/5	3/5	2/5	2/5	2/5	3/5	3/5

Discussion

4.1 Intraocular pressure

Circadian rhythm
This study investigated the effects of the carbonic anhydrase inhibiting eye drops brinzolamide 1% on the circadian rhythm of intraocular pressure in healthy, adult cats. In this study we found a circadian rhythm of the IOP during the placebo period, with a peak value at 9:00h and the lowest value at 18:00h. During the brinzolamide phase, the peak value of the IOP occurred at 0:00h and the lowest value at 6:00h. However, compared to the study of Del Sole et al., (9) the circadian rhythm showed some differences. Del Sole et al. who measured IOP at 9:00h, 12:00h, 15:00h, 18.00h, 21.00h, 0:00h, 3:00h, 6:00h and 9:00h (day 2 of experiment), found peak values during the night from 21:00 till 0:00 hours and the lowest values during the day from 9:00 till 18:00 hours. An explanation for the fact that they found other values, could be the difference in photoperiod to which the animals were exposed. In the study of Del Sole et al. the cats were kept under constant darkness or under a photoperiod of 12 hours of light and 12 hours of darkness. Our study did not have this constant dark-light period. Beside this, in our study night-time measurements were performed under the same artificial light as during day-time measurements. But, the light intensity during the night-time hours was still lower than during day-time hours. In the study of Del Sole et al. measurements during the night were performed under dim red light illumination. However, it is thought that light may not influence the daily variation of the IOP, but that there might be some level of endogenous control (9). More research is needed to check for this endogenous clock-control of intraocular pressure.

Beside this, Del Sole et al. measured with a TonoPen® and we used a TonoVet®. To measure with a TonoPen®, a topical anesthetic is needed. This drop anesthetic could have influenced the IOP. However, measurements performed with a TonoVet®, are less precise when the IOP is high (2,15). But, the cats used in this research were healthy cats, where a high IOP is more unlikely and this was also not measured. The TonoVet® seems to be a good and accurate tonometer of values in the clinically important pressure range and has a low inter-observer variability which is important in a study with more than one observer (6,15).

Application of eye drops temporarily causes a higher viscosity of the tear film. The accuracy of the measurements of the IOP by using a TonoVet® is influenced by this viscosity of the tear film (16). The administration of artificial tears or brinzolamide eye drops in our study could have influenced the IOP if measured when the tear film viscosity had not yet returned to its normal level. Another difference between the study of Del Sole et al., (9) and our study is the fact that they measured the IOP during a 24-hours' period only and our research measured the IOP during 5 days. We also used two sets of measurements of the IOP, in which the IOP of the second set of measurements was significantly lower than the first set. Probably, this second set of measurements is also a reason for the fact that the standard error of measurements of the IOP of Del Sole et al. is higher than our standard error of the second set of measurements.

An explanation for the fact that the IOP at 9:00u during the placebo period was high, could be the fact that the cats just finished their breakfasts and the caretakers went in. The higher IOP value could be correlated with this awakening phase. This circadian rhythm of the IOP must be taken into account in the practice of glaucoma patients. Depending on the time point of measuring, the IOP could be relatively high or low. Therefore, it is important to measure the IOP always at the same time of the day.

IOP reducing effect of brinzolamide

During the brinzolamide period of this study, a statistically significant reduction of the IOP compared to the placebo period was observed at all time points, except at 00:00h. There is no clear explanation for this one non-significant result. In contrast to the results of the underlying study, Gray et al., (14) did not find a significant difference of the mean IOP at any time point during brinzolamide treatment compared with the baseline values. In their discussion they suggested that a larger number of test animals instead of twelve animals may have shown a significant difference. However, in our study ten animals were used and still a significant difference was observed. The most important difference between the study of Gray et al. and the underlying study is the frequency of administration of the medication: Gray et al. administered brinzolamide twice a day and in our study it was administered three times a day. Another possible explanation for the difference is that the study population of Gray et al. consisted of privately owned cats and the medication was administered by the cat's owners. In our study research cats were used and the medication was administered by the researchers. In the discussion of Gray et al. a poor compliance or medication technique by the cat's owners is given as an explanation for the non-significant results. Furthermore, the photoperiod to which the animals were exposed was different for each cat in the research of Gray et al., because the cats were privately-owned. Possibly, the photoperiod could have an effect on the IOP, as mentioned above in the study of Del Sole et al., (9). Furthermore, Gray et al. measured the IOP with an applanation tonometer (TonoPen®) and in our study IOP was measured with a rebound tonometer (TonoVet®), as mentioned in the paragraph before. Beside this, the parameters in the study of Gray et al. were measured for one day and only during day-time hours (from 9:00 till 17:00 hours). In our study the parameters were measured for 5 days and during day-time and night-time hours (from 9:00 till 6:00 hours).

Mc Lellan et al., (13) also found no significant reduction of the IOP in normal cats treated with 1% brinzolamide TID. However, in cats with primary glaucoma they did find a significant reduction of the IOP and the diurnal fluctuations of the IOP were reduced after the treatment with brinzolamide TID. In our study we also found less fluctuations of the IOP during the brinzolamide treatment. From the conference abstract, however, it is not clear what was used as a reference: the contralateral eyes during the medication period or the ipsilateral eyes during the acclimation period. Our study used the placebo-treated, ipsilateral eyes as a control instead of the contralateral eyes. In contrast to Gray et al., (14) McLellan et al. administered brinzolamide three times a day. However, only four animals were studied instead of ten animals in our study. The parameters were measured at 6h intervals in the study of Mc Lellan et al., whereas our study measured the parameters at 3h intervals. Possibly, more measurements on one day, because of the shorter intervals could have influenced the endogenous household of the cats which could have influenced the IOP in our study. A factor that also could have influenced the results is the fact that in our study a second set of measurements of the IOP was used. The other studies did not use a second set of measurements of the IOP.

Systemic effect

In our study, the IOP in the brinzolamide-treated eye was not significantly different from the IOP in the untreated eye, so a reduction of the IOP took also place in the untreated eye. The IOP in the treated eyes was reduced by 14 percent and the IOP in the untreated eyes was reduced by 13 percent. There seemed to be a systemic effect of topically administered brinzolamide. More studies reported a systemic effect of topically administered drugs, like tropicamide and dorzolamide (17,18). Further research is needed to investigate the possible systemic effects of topically administered brinzolamide.

General

In the analysis the measurement of set 2 of the IOP were taken, because set 1 showed much more variation. Probably, the variation of the IOP observed in set 1 was caused by different levels of arousal at the onset of measurements, resulting from their short-distance transportation to the examination room. After the measurements of set 1, the cats were more habituated to the measurements for set 2.

The IOP in the placebo phase seemed to stabilize only after two days. In the first two days, the cats had to get used to the administration of eye drops.

The observers were not completely blind for the medication, the medication vial of Lacriforte® betrayed the type of medication and the administration twice a day of timolol betrayed which of the animals got timolol administered.

4.2 Pupil diameter and light intensity

During the pre-treatment and placebo phase, the pupil diameter was a fixed effect in the linear-mixed effect model of the IOP. However, it seemed to be an incidental finding, because during the brinzolamide phase the pupil diameter was not a fixed effect in the linear-mixed effect model of the IOP. But, at some time points the pupil diameter seemed to follow the same circadian rhythm as the IOP. At these time points the pupil diameter and IOP were concurrently smaller/lower and bigger/higher. Possibly, a larger pupil size causes a higher value of the IOP. This is supported by one study in which a mydriatic drug was administered in cats and thereby the IOP increased (17). More research is needed to a possible influence of the pupil size on the IOP.

In our study a larger pupil diameter was found at some time points during the brinzolamide phase compared to the placebo phase. However, there was a lot of variation of the pupil diameter between the time points, within the time points and between the days. The variation of the pupil diameter between the different time points can be explained by the variation of the light intensity during the day at the different time points. At 12:00h there is a peak value of the light intensity and therefore the pupil diameter at this time point is smaller. It is expected that during the night-time hours in which there is a low light intensity, the pupil diameter is larger than during the day-time hours. However, at 3:00h at night there is a reduction of the pupil diameter. There is no clear explanation for this smaller pupil diameter.

The light intensity follows the same pattern in both the placebo and brinzolamide phase, except at 21:00h. So, the differences in pupil diameter between the placebo and brinzolamide phase cannot be explained by a different light intensity, because the light intensity between the placebo and brinzolamide phase differs only significant at 21:00h. Beside this, carbonic anhydrase inhibitors reportedly do not have an effect on the pupil size (2). There is no clear explanation for the differences in pupil diameter between the placebo and brinzolamide phase. An explanation for a larger pupil diameter could be adrenaline influences. However, it is not clear why these influences would be higher during the brinzolamide phase. Further research is needed on this topic.

Besides all this, measurements of the pupil diameter were difficult, because the pupil diameter could quickly change in the cats. Sometimes during the measurement of the contralateral eye, the pupil diameter in the first eye had already changed.

4.3 Heart rate

No significant differences of the overall heart rate between placebo and brinzolamide were found, but there were some significant differences between the time points. However, there was much variation of the heart rate between the time points and within the time points during the placebo and brinzolamide phase. An explanation for the variation between the time points and within the time points at different days of the heart rate can be arousal or stress of the cats at some time

points. The kennel of the cats was next door to the kennel of research dogs. Sometimes, the dogs were very noisy, for example if someone came in. The cats were used to the barking of the dogs, but while being alone in an examination room this may have caused more stress for the cat than while being with kennel mates. During the placebo phase, the heart rate seemed to follow a circadian rhythm, but during the brinzolamide phase the circadian rhythm was much more difficult to recognize. An explanation for less fluctuations of the heart rate during the brinzolamide phase, could be the habituation to the measurements and handling of the cats during the placebo phase. Due to the habituation, the cats experienced less stress and therefore less fluctuations of the heart rate were observed.

4.4 General points

Our research group of cats consisted of domestic shorthaired cats, which is the most common type of cat in veterinary practice. However, they were of uniform and young age, and in the research population there were brother-brother, sister-brother and sister-sister relations which could have influenced the representativeness for the entire cat population. Furthermore, they were healthy cats without glaucoma. Cats with glaucoma have an increased IOP and the peak value of the IOP is much higher. It is possible that the effects of the medication are different in cats with glaucoma, as suggested by the results of the study of Mc Lellan et al., (15), in which there was only a significant effect of brinzolamide in glaucomatous cats. This is supported by a study in which the administration of a carbonic inhibitor at higher concentrations caused a greater decrease in IOP in Beagles with glaucoma compared with normotensive Beagles (19). Possibly this is also the case with brinzolamide in cats with glaucoma. Further research is needed on this topic.

Beside this, the cats used in this research were young and the age of the cats can have significant influences on the IOP (1). In our study the gender of the cats did not influence the IOP during the brinzolamide and placebo phase. However, in the comparison of the placebo and pre-treatment in our study, there seemed to be a significant difference between genders. This is possibly an incidental finding.

During the measurements of the heart rate a heart murmur was found in one of the male cats. The cat was otherwise asymptomatic. Following clinical and ultrasonographical examination the cardiologist (Diplomate ECVIM-CA Cardiology) concluded that the heart murmur was caused by a dynamic outflow obstruction and by a mild mitral valve regurgitation. Diseases in which these changes occur are hypertrophic obstructive cardiomyopathy or congenital mitral valve dysplasia. The medication of this research project could be administered without any risks. During the research the cat did not show any clinical signs.

All of the twelve cats started to sneeze during the study and one female cat started vomiting at the end of the study. There were no ocular signs of disease. There are no clear reasons to assume that our study was the cause of this illness.

Administration of eye drops seemed to be not applicable in all cases. Two of the twelve cats in this research had to be excluded from the study early, because the cats became unmanageable during the administration of the eye drops. So, a pharmacological therapy is not always an option for the treatment of glaucoma in cats. Ocular irritation after the administration of the eye drops was not observed.

Conclusion

Topical administration of brinzolamide 1% was effective in reducing the intraocular pressure in 10 healthy domestic shorthaired cats. The overall mean IOP decreased by 1.9 mmHg and 14 percent after administration of brinzolamide. No heart rate differences during the brinzolamide phase compared to the placebo phase were found. The pupil diameter of the treated eye during the brinzolamide phase was significantly larger at 4 time points compared to the treated eye of the placebo phase, despite the same light intensity conditions. There is a lack of evidence-based articles and therefore further research is needed in cats with glaucoma.

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