# Effects of topical administration of 2% dorzolamide either alone or in combination with 0.5% timolol on intraocular pressure, pupil diameter and heart rate in clinically healthy cats.

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**Abstract**
OBJECTIVE: To evaluate the effect of topical administration of dorzolamide 2% three times daily, either alone or in combination with topical administration of timolol 0.5% two times daily, on the intraocular pressure (IOP), pupil diameter (PD) and heart rate (HR) in healthy cats.
ANIMALS: Ten clinically healthy domestic shorthair cats.
PROCEDURES: Cats were randomly assigned to groups 1-4 and for each cat the eye medicated was randomly determined. Throughout the experiment IOP, PD with light intensity and HR were measured with 3-h intervals at 09:00h, 12:00h, 15:00h, 18:00h, 21:00h, 00:00h, 03:00h, and 06:00h. From the placebo period onwards, IOP was measured in two consecutive series, $IOP_1$ and $IOP_2$. A 5-day acclimatisation period (days 1-5) was followed by a 5-day placebo period (days 7-11) in which artificial teardrops were administered to one randomly assigned eye and baseline values were obtained. Time points at which eye drops were administered were at 15:30h, 19:45h, 23:30h, 07:30h, and 07:45h. This study was performed in the context of a larger study in which the effect of topical administration of brinzolamide 1% three times daily, either alone or in combination with topical administration of timolol 0.5% two times daily, on the intraocular pressure (IOP) in healthy cats was also evaluated. Medication protocols in groups 1-4 were rotated according to a Latin square schedule. The medication protocols were: dorzolamide 2% eye drops q8h (A), brinzolamide 1% eye drops q8h (B), dorzolamide 2% eye drops q8h + timolol 0.5% eye drops q12h (C), and brinzolamide 1% eye drops q8h + timolol 0.5% eye drops q12h (D). During the treatment periods, artificial teardrops were administered to the fellow eye at all medication time points and to the medicated eye at time points 19:45h and 07:45h when the treatment did not include timolol. The treatment periods of five days were followed by a washout period of three days.
RESULTS: Mean $IOP_2$ during the placebo period was 14.2 ± 0.1 mmHg. Mean $IOP_2$ decreased significantly ($p < 0.001$) in treatment periods A and C compared to IOP values of the placebo period. There was a significant ($p = 0.009$) decrease in mean $IOP_2$ in treatment C compared to mean $IOP_2$ of treatment A. Treatment C caused a significant ($p < 0.001$) decrease in PD of the treated eye, but did not cause a significant change in HR.
CONCLUSION: The results of the present study suggest that in healthy cats the topical administration of dorzolamide 2% TID in combination with timolol 0.5% BID has a significantly greater IOP lowering effect than the administration of dorzolamide 2% TID alone. A study on the effect of the combined administration of dorzolamide and timolol in cats with glaucoma is warranted.

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**Keywords:** cats, glaucoma, intraocular pressure, dorzolamide, timolol

## Contents

## Introduction

Glaucoma is one of the most common emergency cases in ophthalmology in cats[1]. Feline glaucomas consist of a group of diseases that are characterised by the progressive death of retinal ganglion cells and their axons (i.e., optic nerve fibres), which will eventually lead to blindness[1]. Glaucomas can be divided in several types by their stage of disease and aetiology. Glaucomas can arise primary or secondary[1]. Secondary glaucoma is caused by pre-existing intraocular diseases and is the most common type of feline glaucoma. These pre-existing intraocular diseases include anterior uveitis, lens cataract or lens luxation, intraocular neoplasms, hyphaema and aqueous humour misdirection syndrome, of which anterior uveitis is the most frequent underlying disorder[1].

An increased intraocular pressure (IOP) is considered to be one of the main clinical features of glaucoma. Cats with glaucoma rarely show signs of ocular discomfort unless there is a markedly elevated IOP.[1,2] In addition, this increased IOP will eventually lead to irreversible blindness in cats[1]. IOP values show significant 24-h variations, which are due to a circadian rhythm. This circadian rhythm reportedly has a peak IOP between 21:00h and 00:00h which corresponds to the dark period in which cats are usually active[3]. The IOP results from the balance between the aqueous humour (AH) production and outflow. An increase in IOP arises if the aqueous outflow is decreased or, theoretically, if there is an increase in aqueous production. There are several ways in which an elevated IOP can be decreased: by decreasing AH production, increasing the AH outflow, or both[1]. AH production can be reduced by inhibiting the enzyme carbonic anhydrase, which is present in the non-pigmented epithelium of the ciliary body[4]. Carbonic anhydrase catalyses the synthesis of $HCO_3^-$ + $H^+$ from $H_2O$ + $CO_2$, $HCO_3^-$ passes from the non-pigmented epithelium into the posterior chamber in which it will attract $Na^+$ and thereby create an osmotic gradient, causing a passive flow of water into the posterior chamber and thereby forming AH. Carbonic anhydrase inhibitors (CAIs) reduce the amount of $HCO_3^-$, and thereby of AH, being formed[5]. CAIs can be administered systemically or topically. Topical administration is preferred due to the fact that systemic CAIs are associated with several side effects and complications in cats[6]. Eye drops that are being used in both animals and humans include the CAIs dorzolamide 2% and brinzolamide 1% with or without the non-selective beta-adrenergic blocking agent timolol 0.5%. Timolol is an additional agent that can reduce the AH production and thereby decrease the IOP. Timolol is a beta-blocker that binds to beta-adrenergic receptors of the ciliary body, thereby inhibiting cyclic AMP synthesis and reducing AH production[7]. Dorzolamide 2%, brinzolamide 1%, and timolol 0.5% are human topical glaucoma medications that are frequently prescribed for cats. Their effects on the IOP in cats have been studied to a limited extent. It was suggested that administration of dorzolamide 2% three times daily had more IOP-lowering effect than administering it two times daily[8]. When applied twice daily, the mean IOP was lowered by 2.3 mmHg (18%) in eight healthy cats and lowered by 2.1 mmHg (11%) in a different study with six healthy cats[8,9]. In another study in which dorzolamide 2% was applied three times daily, the mean IOP was lowered by 3.8 mmHg (26%) in fifteen healthy cats[10]. Topical administration of a single dose of timolol 0.5% resulted in a reduction of the IOP in the treated and the contralateral eye[11]. In cats the effect of timolol 0.5% on the IOP reduction reportedly was 22% in the treated eye and 16% in the contralateral eye[11]. Timolol 0.5% should not be applied more than twice daily due to the emergence of severe side effects. These side effects include bradycardia, hypotension, and bronchospasm, depending on the administered dose, when the drug is systemically absorbed[1]. Dietrich et al. examined the effect on the IOP when administering dorzolamide 2% in combination with timolol 0.5% twice daily in six healthy cats and the effect of dorzolamide 2% alone three times daily in six healthy cats. Both medication protocols had a significant IOP lowering effect, but the

combination of the two drugs did not result in a significantly greater IOP decrease than dorzolamide 2% applied three times daily alone[8]. The effect of timolol 0.5% administered twice daily in combination with dorzolamide 2% three times daily has not yet been studied. The objectives of the study reported here were to investigate the effects of topically administering the carbonic anhydrase inhibiting drug dorzolamide 2% three times daily and the B-blocking drug timolol 0.5% two times daily on the circadian intraocular pressure in healthy cats. We hypothesized that in healthy adult cats, the topical administration of dorzolamide 2% three times daily and timolol 0.5% two times daily would result in a significantly higher decrease in IOP than administration of dorzolamide 2% three times daily alone.

## Material and methods

*Animals:*

Twelve clinically healthy domestic shorthair cats from the clinical teaching colony of Utrecht University Department of Clinical Sciences of Companion Animals were included. The cohort consisted of six neutered males and six spayed females, in the age of 1.5 years, from six different litters of which respectively two, three and four cats were from the same litter. Male and female cats were housed separately in a temperature-controlled environment and were exposed to consistent room lighting conditions. These animals were not used for education during this project. Prior to the study the eyes of the cats were examined by a board-certified veterinary ophthalmologist to confirm ocular health and at the end of each medication period the ophthalmologist monitored ocular irritation using a modified Hackett-McDonald semi-quantitative scale. The study was approved by the Animal Welfare Body Utrecht.

**Table 1**- Overview of the composition of each group of cats (1-4). F(=female), M(=male), OD(=right), and OS(=left).

| Group | Cat number | Cat names and sex | Treated eye |
|---|---|---|---|
| 1 | 1 | Lolita (F) | OD |
| 1 | 2 | Carlito (M) | OD |
| 1 | 3 | Filippe (M) | OD |
| 2 | 4 | Dantae (M) | OS |
| 2 | 5 | Bonita (F) | OD |
| 2 | 6 | Dali (M) | OS |
| 3 | 7 | Balthasar (M) | OD |
| 3 | 8 | Carmelita (F) | OD |
| 3 | 9 | Catalina (F) | OS |
| 4 | 10 | Enrique (M) | OS |
| 4 | 11 | Rosalia (F) | OS |
| 4 | 12 | Julieta (F) | OD |

Medication protocols: A: dorzolamide 2% eye drops q8h, B: brinzolamide 1% eye drops q8h, C: dorzolamide 2% eye drops q8h + timolol 0.5% eye drops q12h, and D: brinzolamide 1% eye drops q8h + timolol 0.5% eye drops q12h. The four groups received the medication protocols in different orders, group 1: A, B, C, D, group 2: B, C, D, A, group 3: C, D, A, B, and group 4: D, A, B, C.

*Methods:*

This was a prospective, double-blind study. Four students, divided in two groups, administered the medication, against which they were blinded, and performed the measurements. Twelve clinically healthy cats were divided in four different medication groups (1-4) by using a Random Team Generator. These groups were all exposed to the four medication protocols by a rotating schedule (Latin square) and for each cat the treated and control eye were determined by the flip-a-coin method. Throughout the experiment IOP, pupil diameter (PD) with light intensity and heart rate (HR) were measured with 3-h intervals at 09:00h, 12:00h, 15:00h, 18:00h, 21:00h, 00:00h, 03:00h, and 06:00h. During the first five days of the project, the pre-treatment or acclimatisation phase, the cats were acclimatised to the measurements. IOP was measured using a rebound tonometer (TonoVet, ICare, Vantaa, Finland), PD was measured using digital calipers (kwb Germany GmbH, Stuhr, Germany), light intensity was measured using a digital lux meter (Showtec digital luxmeter, MS6610,

Poway, California, USA) and HR was measured using a phonendoscope for thoracic auscultation (3M™ Littmann stethoscope, Classic II S.E., St. Paul, USA). From the placebo period onwards, for each eye two series of three IOP measurements were performed in a sequence of OD-OS-OD-OS, and the mean of these three values were termed $IOP_1$ (for the first series) and $IOP_2$ (for the second series). After the pre-treatment phase, one day of rest was followed by a five-day placebo period in which an artificial tear drop (Lacriforte®, AST farma, Oudewater, The Netherlands**)** was administered to the randomly assigned eye at 15:30h, 19:45h, 23:30h, 07:30h, and 07:45h. The other eye did not receive any medication. One day of rest followed the placebo period. After the pre-treatment and placebo period, four treatment periods followed. Each treatment period took five days, followed by a washout period of three days during which no medication was administered. At the last day of each washout period, IOP, PD with light intensity and HR measurements were measured. In protocol A, dorzolamide 2% eye drops (Dorzolamide® 20 mg/ml, Cetrafarm B.V., Etten-Leur, The Netherlands**)** were administered every eight hours to one eye, while an artificial tear was administered every twelve hours to the same eye and five times daily to the other eye. In protocol B, brinzolamide 1% eye drops (Azopt®, Alcon, Camberley, UK) were administered every eight hours to one eye, while an artificial tear was administered every twelve hours to the same eye and five times daily to the other eye. In protocol C, dorzolamide 2% eye drops were administered every eight hours to one eye in combination with timolol 0.5% eye drops (Timolol® Sandoz 5 mg/ml, Sandoz, Almere, Nederland) which were administered every twelve hours to the same eye, while an artificial tear was administered five times daily to the other eye. In protocol D, brinzolamide 1% eye drops were administered every eight hours to one eye in combination with timolol 0.5% eye drops which were administered every twelve hours to the same eye, while an artificial tear was administered five times daily to the other eye.

In summary, during a continuous period of 42 days every three hours the IOP, PD with light intensity and HR were measured with the exception of 1 day after the pre-treatment phase, 1 day after the placebo phase and the first two days of the three-day washout period following each of the four medication phases. There was no washout period after day 42.

| Time point | Measurements | Medication (for the medicated eye; the contralateral eye received an artificial tear drop for each medication) |
|---|---|---|
| 09:00h | IOP, PD, LI, HR | |
| 12:00h | IOP, PD, LI, HR | |
| 15:00h | IOP, PD, LI, HR | |
| 15:30h | | Dorzolamide (A/C) or Brinzolamide (B/D) |
| 18:00h | IOP, PD, LI, HR | |
| 19:45h | | Timolol (C/D) or Artificial tear drop (A/B) |
| 21:00h | IOP, PD, LI, HR | |
| 23:30h | | Dorzolamide (A/C) or Brinzolamide (B/D) |
| 00:00h | IOP, PD, LI, HR | |
| 03:00h | IOP, PD, LI, HR | |

| | | |
|---|---|---|
| 06:00h | IOP, PD, LI, HR | |
| 07:30h | | Dorzolamide (A/C) or Brinzolamide (B/D) |
| 07:45h | | Timolol (C/D) or Artificial tear drop (A/B) |

**Table 2–** Overview of the times at which daily measurements of intraocular pressure (IOP), pupil diameter (PD), ambient light intensity at eye level (LI) and heart rate (HR) were performed and medication was administered to 12 healthy cats. Four medication protocols, A-D, A: dorzolamide 2% eye drops q8h, B: brinzolamide 1% eye drops q8h, C: dorzolamide 2% eye drops q8h + timolol 0.5% eye drops q12h, and D: brinzolamide 1% eye drops q8h + timolol 0.5% eye drops q12h, were applied.

*Statistical analysis:*
Methodology: prospective, blinded, paired observational study.
Power analysis: With repeated measurements in ten cats, P (alpha) set at < 0.05 and a power set at 80%, the minimum IOP lowering effect which can be detected statistically is 0.9 mmHg.
Statistical analysis of the data was performed using RStudio (Version 0.99.903 – © 2009-2016 RStudio, Inc.). All data are shown as mean ± SE, except where indicated otherwise. The values of the treated eye were compared with the baseline values of the treated eye, which were obtained in the placebo period. A linear mixed-effect model was used for statistical analysis which was well suited for the longitudinal data obtained in this research project. Animal was considered as a random factor in the model. Fixed factors in the model were day, time, and sex. IOP measurement of a male cat at 09:00h on day 1 of a treatment period was considered the baseline value. A Tukey test was used following the lme analysis to test overall, time and day trends of IOP in the different treatment periods. A multiple regression analysis is performed as well to test the moderating effect of light intensity on PD and the effect of the treatment itself. P (alpha) set at < 0.05 was considered statistically significant.

## Results

*Animal numbers and dates*

Twelve clinically healthy cats were included in the study. Two cats were removed from the study after the placebo period due to the fact that it was not possible to administer the eye drops. These two cats, number 7 and 12, belonged to group three and four and their measured values were excluded from the research project. The very first (09:00h) measurements of the placebo period were not performed due to circumstances. As a practical solution the lacking 09:00h data of day 1 were replaced by 09:00h measurements of day 2 of the placebo period.

*Acclimatisation data*

Mean $IOP_1$ per day of the eye to be treated in the pre-treatment period is depicted in figure 1. The mean $IOP_1$ values of each day of the eye to be treated were as follows: day1=18.0 ± 0.4 mmHg, day2=16.5 ± 0.4 mmHg, day3=15.1 ± 0.4 mmHg, day4=15.2 ± 0.4 mmHg, and day5=15.2 ± 0.3 mmHg. $IOP_1$ values during day 1 and day 2 showed higher $IOP_1$ values and greater dispersion when compared to days 3-5 of the pre-treatment period. $IOP_1$ results from day 1 and 2 did not include the measurements of all cats due to the fact that the measurements took a long time at the beginning of the present study. As can be seen in Table 3, a significantly higher mean $IOP_1$ during day 1 and day 2 of the pre-treatment period was found when compared with days 3-5 of this period ($p < 0.05$). Mean $IOP_1$ in the eyes to be treated of day 3-5 was 15.2 ± 0.2 mmHg (Table 10). Mean $IOP_1$ of days 3-5 in OD was 16.4 ± 0.4 mmHg and mean $IOP_1$ in OS was 13.5 ± 0.2 mmHg, IOP in OS being significantly lower than in OD ($p < 0.001$). $IOP_1$ values were different for different time points during the day, with a mean $IOP_1$ of days 3-5 of 18.1 ± 0.5 mmHg at 09:00h, 15.9 ± 0.6 mmHg at 12:00h, 15.2 ± 0.5 mmHg at 15:00h, 13.6 ± 0.5 mmHg at 18:00h, 14.9 ± 0.6 mmHg at 21:00h, 15.2 ± 0.7 mmHg at 00:00h, 14.3 ± 0.5 mmHg at 03:00h and 14.1 ± 0.4 mmHg at 06:00h in the eye to be treated (Figure 2). The mean PD of the eye to be treated for the clinically healthy cats in the pre-treatment period of days 3-5 was 4.8 ± 0.1 mm and the mean HR of days 3-5 of the pre-treatment period was 149 ± 1.5 bpm (Table 10).

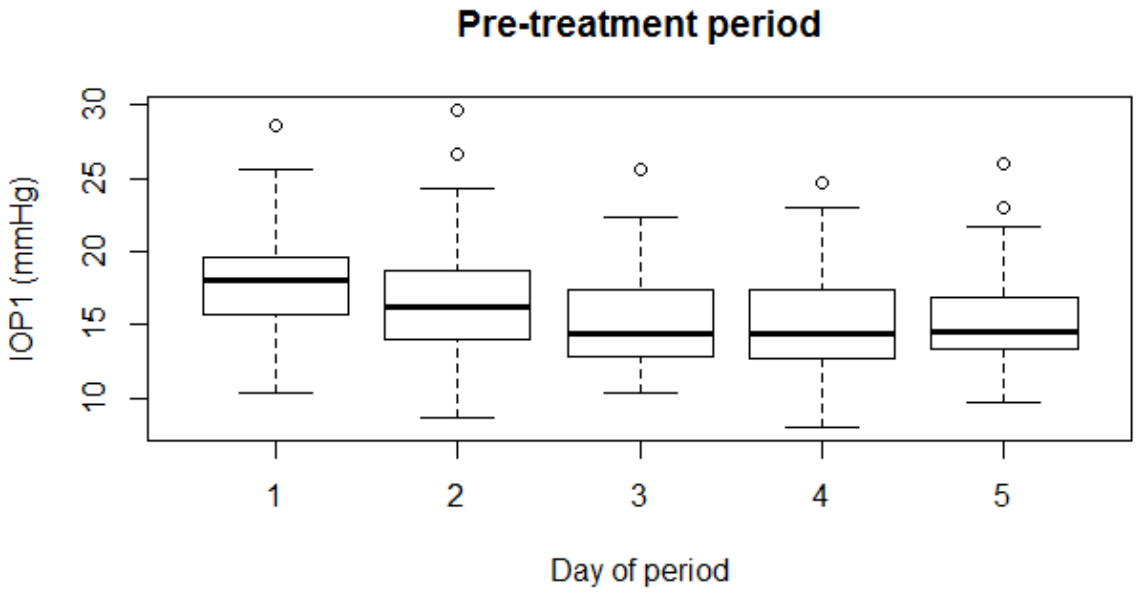


**Figure 1-** Mean 24-hours' intraocular pressure ($IOP_1$) in mmHg in the eye to be medically treated on each day of the pre-treatment period in 10 healthy cats. Mean $IOP_1$ values of day 1 and 2 were significantly higher than mean $IOP_1$ values of days 3-5 (Table 3).

**Table 3–** Pre-treatment period, days on which $IOP_1$ of the eyes to be treated of 10 healthy cats differed significantly ($p < 0.05$). Estimate= the value of the $IOP_1$ of the first time point minus the $IOP_1$ of the second time point, and SE= standard error.

| Days compared | estimate | SE | p.value |
|---|---|---|---|
| 1 - 3 | 2.786 | 0.478 | <.0001 |
| 1 - 4 | 2.735 | 0.478 | <.0001 |
| 1 - 5 | 2.706 | 0.478 | <.0001 |
| 2 - 3 | 1.392 | 0.469 | 0.0266 |
| 2 - 4 | 1.341 | 0.469 | 0.0363 |
| 2 - 5 | 1.312 | 0.469 | 0.0433 |

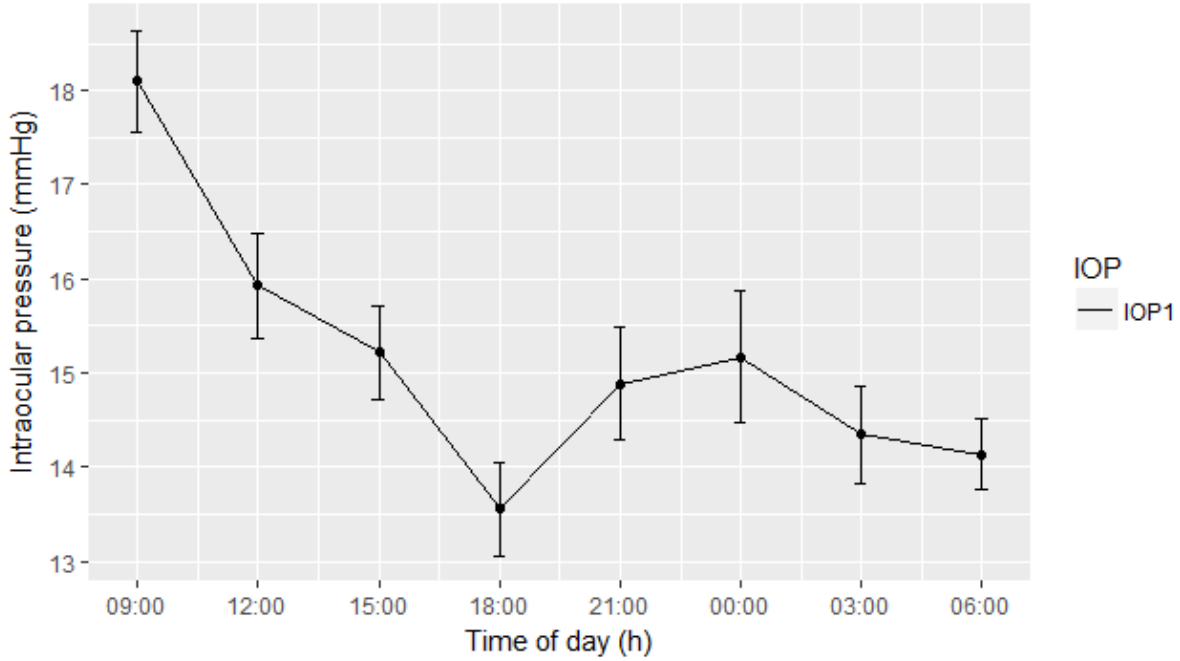


**Figure 2–** Mean intraocular pressure ($IOP_1$) in mmHg during the day in the eye to be medically treated during day 3-5 of the pre-treatment period in 10 healthy cats. Standard errors of each IOP value were depicted in this figure with bars.

*Baseline data*

Baseline data was obtained during the placebo period. Mean $IOP_1$ and $IOP_2$ per day of the eye to be treated in the placebo period are depicted in figure 3a and 3b, respectively. Both $IOP_1$ and $IOP_2$ were significantly higher at day 1 and 2 than at day 4 and 5 ($p < 0.05$) (Figure 3a-b, Table 4a-b). The mean IOP of the first series of measurements ($IOP_1$) of the treated eye for the clinically healthy cats was 14.9 ± 0.2 mmHg and the $IOP_1$ value differed during the day (Figure 4). Mean IOPs at all time points were compared and the time points at which IOP differed significant can be seen in Table 5a. Mean $IOP_1$ values were significantly higher at 09:00h and 12:00h than at 15:00h, 18:00h and 03:00h. The mean $IOP_1$ value at 21:00h was significantly higher than at 18:00h ($p < 0.05$). Mean $IOP_1$ in OD was 16.3 ± 0.6 mmHg and mean $IOP_1$ in OS was 13.3 ± 0.1 mmHg, $IOP_1$ in OS was significantly lower than in OD ($p < 0.001$). Mean $IOP_2$ in OD was 15.3 ± 0.5 mmHg and mean $IOP_2$ in OS was 12.9 ± 0.2 mmHg, $IOP_2$ in OS was significantly lower than $IOP_2$ in OD ($p < 0.001$). Mean $IOP_2$ of the eye that received artificial tears in the placebo period was 14.2 ± 0.1 mmHg and in the fellow eye $IOP_2$ was 14.0 ± 0.1 mmHg, which did not differ significantly ($p = 0.2166$). There was a significant ($p < 0.001$) difference between $IOP_1$ and $IOP_2$ in the medicated eye. $IOP_2$ values differed

during the day (Figure 4) of which the time points which differ significant can be seen in Table 5b. The $IOP_2$ value at 09:00h was significantly higher than at 15:00h, 18:00h, 03:00h and 06:00h during the placebo period and the $IOP_2$ value at 12:00h was significantly than at 18:00h. The mean PD for both the treated eye and the untreated eye for the clinically healthy cats in the placebo period was 5.1 ± 0.1 mm and the mean HR was 152 ± 1.2 bpm (Table 10).

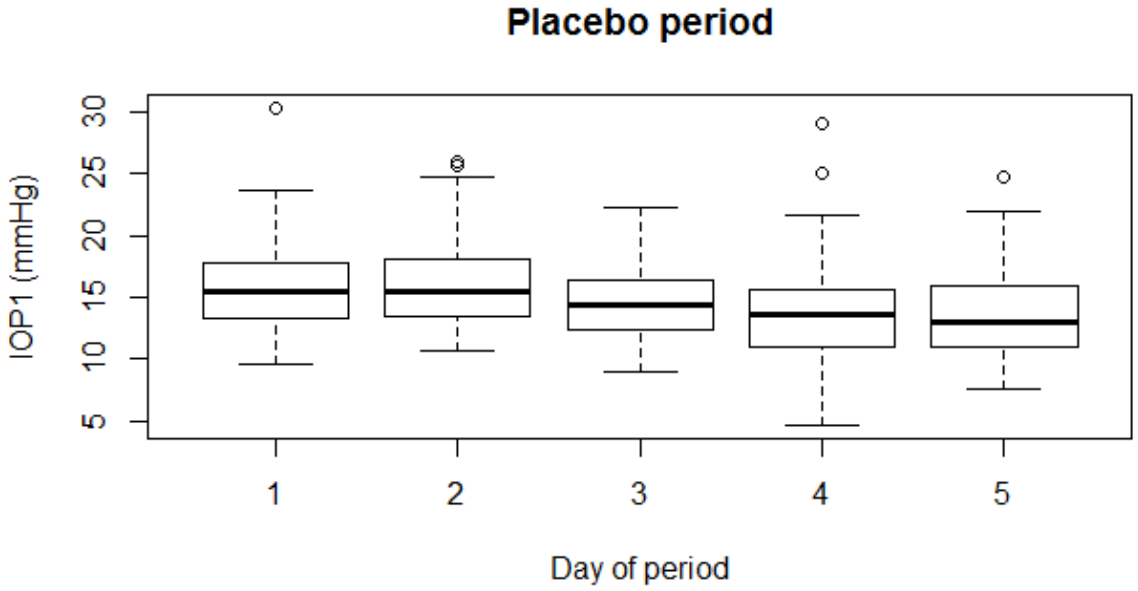


**Figure 3a-** Mean $IOP_1$ in mmHg of the placebo-treated eyes of 10 healthy cats on each day of the placebo period. $IOP_1$ of day 1 was significantly higher than $IOP_1$ of day 4 and 5, and $IOP_1$ of day 2 was significantly higher than $IOP_1$ of days 3-5 (Table 4a).

**Table 4a–** Placebo period, days on which $IOP_1$ of the treated eyes of 10 healthy cats differed significantly ($p < 0.05$). Estimate= the value of the $IOP_1$ of the first time point minus the $IOP_1$ of the second time point, and SE= standard error.

| Days compared | estimate | SE | p.value |
|---|---|---|---|
| 1 - 4 | 1.863 | 0.472 | 0.0013 |
| 1 - 5 | 2.121 | 0.472 | 0.0001 |
| 2 - 3 | 1.572 | 0.472 | 0.0123 |
| 2 - 4 | 2.234 | 0.472 | <.0001 |
| 2 - 5 | 2.492 | 0.472 | <.0001 |

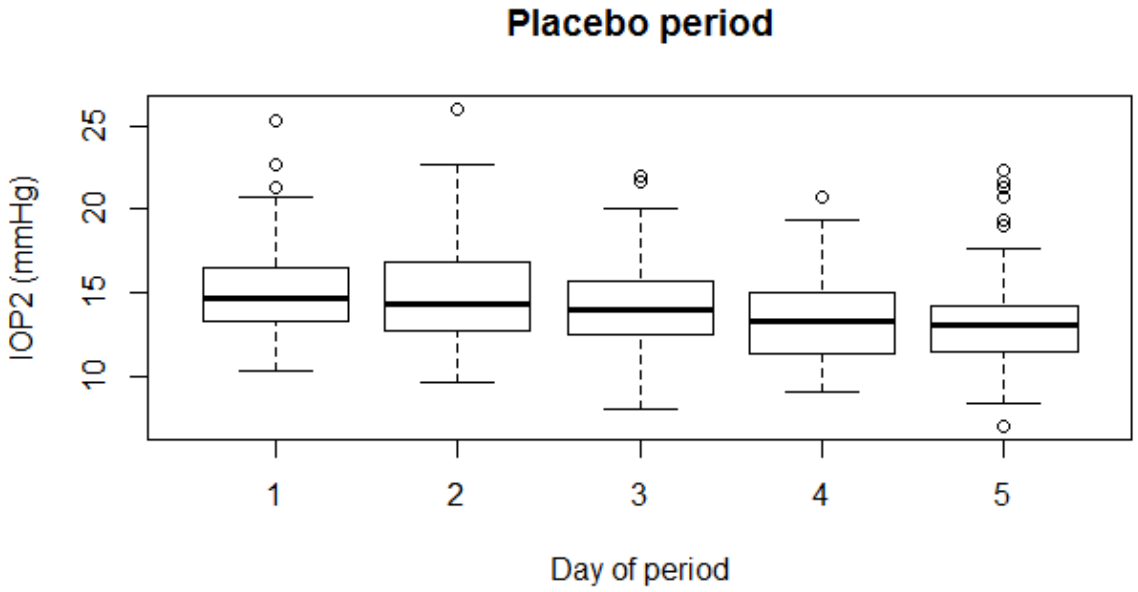


**Figure 3b-** Mean $IOP_2$ in mmHg of the placebo-treated eyes of 10 healthy cats on each day of the placebo period. $IOP_2$ of day 1 and 2 was significantly higher than $IOP_2$ of day 4 and 5 (Table 4b).

**Table 4b–** Placebo period, days on which $IOP_2$ of the treated eyes of 10 healthy cats differed significantly ($p < 0.05$). Estimate= the value of the $IOP_2$ of the first time point minus the $IOP_2$ of the second time point, and SE= standard error.

| Days compared | estimate | SE | p.value |
|---|---|---|---|
| 1 - 4 | 1.837 | 0.403 | 0.0001 |
| 1 - 5 | 1.916 | 0.403 | <.0001 |
| 2 - 4 | 1.587 | 0.403 | 0.0013 |
| 2 - 5 | 1.666 | 0.403 | 0.0006 |

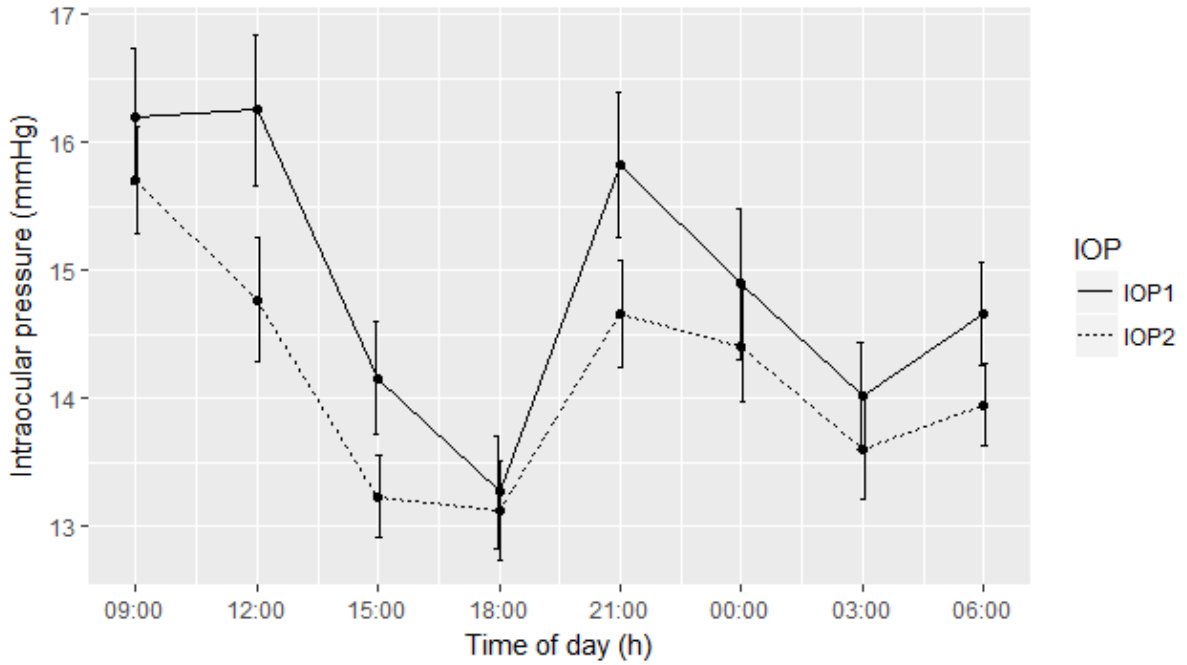


**Figure 4–** Mean intraocular pressure in the placebo-treated eye of 10 healthy cats (two consecutive measurements, $IOP_1$ and $IOP_2$, in mmHg) on different times of the day during the placebo period. Standard errors of each IOP value were depicted in this figure with bars.

**Table 5a–** Placebo period, time points at which intraocular pressure ($IOP_1$) in the placebo-treated eye of 10 healthy cats differed significantly ($p < 0.05$). Estimate= the value of the $IOP_1$ of the first time point minus the $IOP_1$ of the second time point, and SE= standard error.

| Time points compared | estimate | SE | p.value |
|---|---|---|---|
| 1 - 3 | 2.045 | 0.570 | 0.0089 |
| 1 - 4 | 2.939 | 0.570 | <.0001 |
| 1 - 7 | 2.185 | 0.570 | 0.0036 |
| 2 - 3 | 2.093 | 0.595 | 0.0114 |
| 2 - 4 | 2.986 | 0.595 | <.0001 |
| 2 - 7 | 2.233 | 0.595 | 0.0049 |
| 4 - 5 | -2.560 | 0.595 | 0.0006 |

**Table 5b–** Placebo period, time points at which intraocular pressure ($IOP_2$) in the placebo-treated eye of 10 healthy cats differed significantly ($p < 0.05$). Estimate= the value of the $IOP_2$ of the first time point minus the $IOP_2$ of the second time point, and SE= standard error.

| Time points compared | estimate | SE | p.value |
|---|---|---|---|
| 1 - 3 | 2.477 | 0.485 | <.0001 |
| 1 - 4 | 2.585 | 0.485 | <.0001 |
| 1 - 7 | 2.117 | 0.485 | 0.0004 |
| 1 - 8 | 1.763 | 0.485 | 0.0075 |
| 2 - 4 | 1.648 | 0.506 | 0.0268 |

*Medication periods data*

After the washout period of a medication period it was noticed that there was still an influence on the IOP of the last medication, the cats were not yet on their baseline. After a treatment period which included timolol, a difference in PD between the treated and the untreated eye was noticed. Comparing the baseline IOP value from the placebo period with the IOP value of

the day before treatment, a significant ($p < 0.001$) decrease in IOP was observed. Mean $IOP_2$ at day 0, the day before medication protocol C (dorzolamide 2% eye drops q8h + timolol 0.5% eye drops q12h) was administered, was 12.5 ± 0.3 mmHg and mean $IOP_2$ during the placebo period was 14.2 ± 0.1 mmHg. Mean $IOP_2$ was significantly lower at day 0 than during the placebo period ($p < 0.001$). For each treatment period, mean $IOP_1$ and $IOP_2$ of the treated eye and their standard deviations (SD) are summarized in Table 6. SD of $IOP_1$ was larger than SD of $IOP_2$. The mean $IOP_1$ (± SE) values of the treated eye during treatment A (dorzolamide 2% eye drops q8h) and C (dorzolamide 2% eye drops q8h + timolol 0.5% eye drops q12h) were respectively 12.5 ± 0.1 mmHg and 12.0 ± 0.1 mmHg. The mean overall decrease of $IOP_1$ in treatment A and C were respectively 2.5 mmHg and 2.9 mmHg, a decrease of 16.5 % and a decrease of 19.6 %. In both treatment A and treatment C $IOP_1$ was significantly decreased as compared to the baseline values from the placebo period ($P < 0.001$). $IOP_1$ of treatment C was significantly more decreased than $IOP_1$ of treatment A ($P = 0.011$).

The mean $IOP_1$ and $IOP_2$ values during the day during treatment C can be seen in Figure 5. The $IOP_1$ values of treatment C were significantly higher at 21:00h and 00:00h than at 09:00h, 12:00h , 18:00h, 03:00h and 06:00h. The $IOP_1$ value at 21:00h was also significantly higher than at 15:00h (Table 7a). The $IOP_2$ values were significantly higher at 21:00h and 00:00h than at 09:00h, 18:00h and 03:00h during treatment C (Table 7b). The $IOP_2$ percentage drop at each time point of treatment C compared with the placebo period can be seen in Table 8.

**Table 6-** Mean intraocular pressure in mmHg from measurement series 1 ($IOP_1$) and 2 ($IOP_2$) during six treatment periods in the treated eye of 10 healthy cats. PT=pre-treatment, P=placebo period, A=dorzolamide 2% eye drops q8h, B=brinzolamide 1% eye drops q8h, C=dorzolamide 2% eye drops q8h + timolol 0.5% eye drops q12h, and D=brinzolamide 1% eye drops q8h + timolol 0.5% eye drops q12h. NA=not available, which was due to the fact that no $IOP_2$ values were obtained during the PT period.

| Treatment | N($IOP_1$/$IOP_2$) | mean($IOP_1$/$IOP_2$) | SD($IOP_1$/$IOP_2$) | SE($IOP_1$/$IOP_2$) |
|---|---|---|---|---|
| PT | 395/0 | 16.0/NA | 3.513/NA | 0.177/NA |
| P | 410/410 | 14.9/14.2 | 3.747/2.976 | 0.185/0.147 |
| A | 396/396 | 12.4/11.8 | 2.949/2.482 | 0.148/0.125 |
| B | 400 /400 | 12.7/11.9 | 2.696/2.367 | 0.135/0.118 |
| C | 400/399 | 12.0/11.4 | 2.489/2.528 | 0.124/0.127 |
| D | 400/400 | 12.2/11.3 | 2.716/2.384 | 0.136/0.119 |

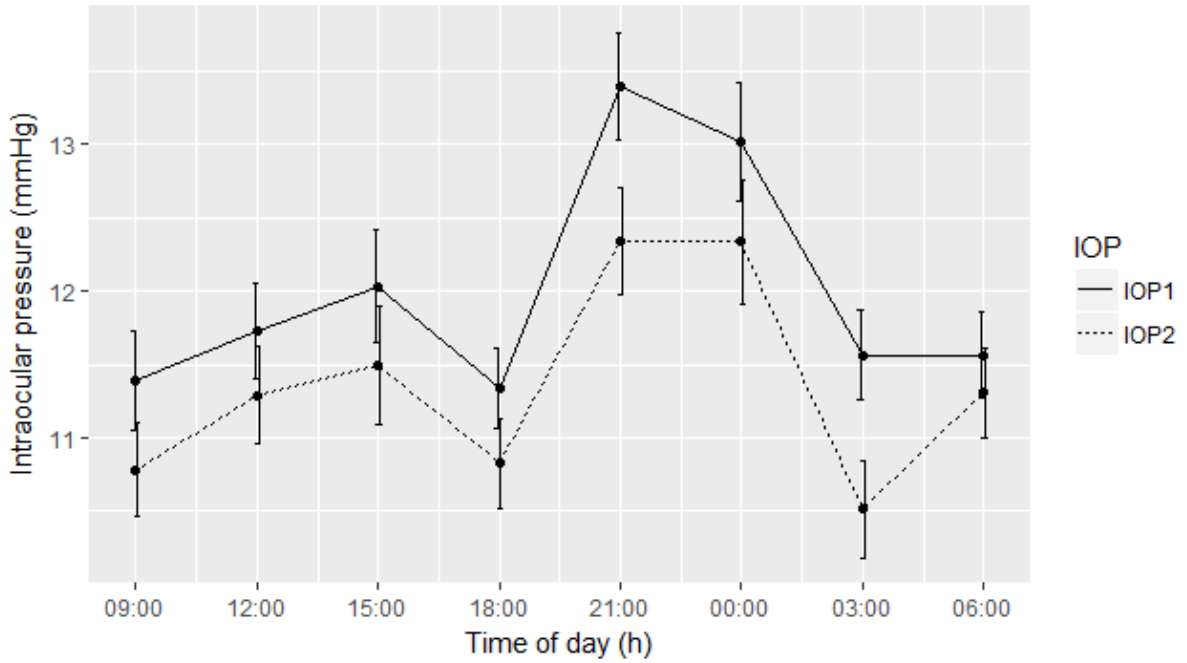


**Figure 5–** Mean intraocular pressure (measured twice: $IOP_1$ and $IOP_2$, in mmHg) in the treated eye of 10 healthy cats on different times of the day following topical administration of dorzolamide 2% eye drops TID and timolol 0.5% eye drops BID on 5 consecutive days (treatment period C). Standard errors of each IOP value were depicted in this figure with bars.

**Table 7a–** Treatment period C, time points at which intraocular pressure ($IOP_1$) in the treated eye of 10 healthy cats differed significantly ($p < 0.05$). Estimate= the value of the $IOP_1$ of the first time point minus the $IOP_1$ of the second time point, and SE= standard error.

| Time point | estimate | SE | p.value |
|---|---|---|---|
| 1 - 5 | -2.006 | 0.416 | 0.0001 |
| 1 - 6 | -1.627 | 0.416 | 0.0027 |
| 2 - 5 | -1.666 | 0.416 | 0.0019 |
| 2 - 6 | -1.286 | 0.416 | 0.0439 |
| 3 - 5 | -1.366 | 0.416 | 0.0245 |
| 4 - 5 | -2.060 | 0.416 | <.0001 |
| 4 - 6 | -1.680 | 0.416 | 0.0017 |
| 5 - 7 | 1.833 | 0.416 | 0.0004 |
| 5 - 8 | 1.834 | 0.416 | 0.0004 |
| 6 - 7 | 1.453 | 0.416 | 0.0123 |
| 6 - 8 | 1.454 | 0.416 | 0.0123 |

**Table 7b–** Treatment period C, time points at which intraocular pressure ($IOP_2$) in the treated eye of 10 healthy cats differed significantly ($p < 0.05$). Estimate= the value of the $IOP_2$ of the first time point minus the $IOP_2$ of the second time point, and SE= standard error.

| Time point | estimate | SE | p.value |
|---|---|---|---|
| 1 - 5 | -1.560 | 0.425 | 0.0068 |
| 1 - 6 | -1.559 | 0.425 | 0.0068 |
| 4 - 5 | -1.513 | 0.425 | 0.0099 |
| 4 - 6 | -1.513 | 0.425 | 0.0100 |
| 5 - 7 | 1.826 | 0.425 | 0.0006 |
| 6 - 7 | 1.826 | 0.425 | 0.0006 |

**Table 8–** Mean $IOP_2$ values during the day for treatment period P and C. P=placebo period and C=dorzolamide 2% eye drops q8h + timolol 0.5% eye drops q12h. P-C value is mean $IOP_2$ during treatment P minus mean $IOP_2$ during treatment C. P-C in %: percentage drop of $IOP_2$ during treatment C when compared to $IOP_2$ during treatment P.

| Treatment | 09:00 | 12:00 | 15:00 | 18:00 | 21:00 | 00:00 | 03:00 | 06:00 |
|---|---|---|---|---|---|---|---|---|
| P | 15.7 | 14.8 | 13.2 | 13.1 | 14.7 | 14.4 | 13.6 | 13.9 |
| C | 10.8 | 11.3 | 11.5 | 10.8 | 12.3 | 12.3 | 10.5 | 11.3 |
| P-C | 4.9 | 3.5 | 1.7 | 2.4 | 2.4 | 2.1 | 3.1 | 2.6 |
| P-C in % | 31.2% | 23.6% | 12.9% | 18.5% | 16.3% | 14.6% | 22.8% | 18.7% |

The mean $IOP_2$ values of the treated eye during treatment A and C were respectively 11.8 ± 0.1 mmHg and 11.4 ± 0.1 mmHg (Table 10). The mean overall decrease in $IOP_2$ during treatments A and C were 2.4 mmHg (16.9%) and 2.8 mmHg (19.7%), respectively. In both treatment A and treatment C $IOP_2$ was significantly decreased when compared with baseline values from the placebo period ($p < 0.001$). $IOP_2$ was significantly more decreased during treatment C than during treatment A ($p = 0.009$). The mean $IOP_2$ values of the fellow eye during treatment A and C were respectively 11.6 ± 0.1 mmHg and 11.1 ± 0.1 mmHg (Table 10). In both treatment A and treatment C $IOP_2$ was also significantly decreased in the untreated eye when compared with baseline values of the untreated eye from the placebo period ($p < 0.001$). $IOP_2$ was also significantly more decreased in the untreated eye during treatment C than during treatment A ($p = 0.001$). $IOP_2$ values of the treated and untreated eyes during treatment A and C did not differ significantly (respectively $p = 0.2314$, $p = 0.0519$). The $IOP_2$ decrease in the treated eye during treatment A and C when compared to the placebo period can be seen in Figure 6.

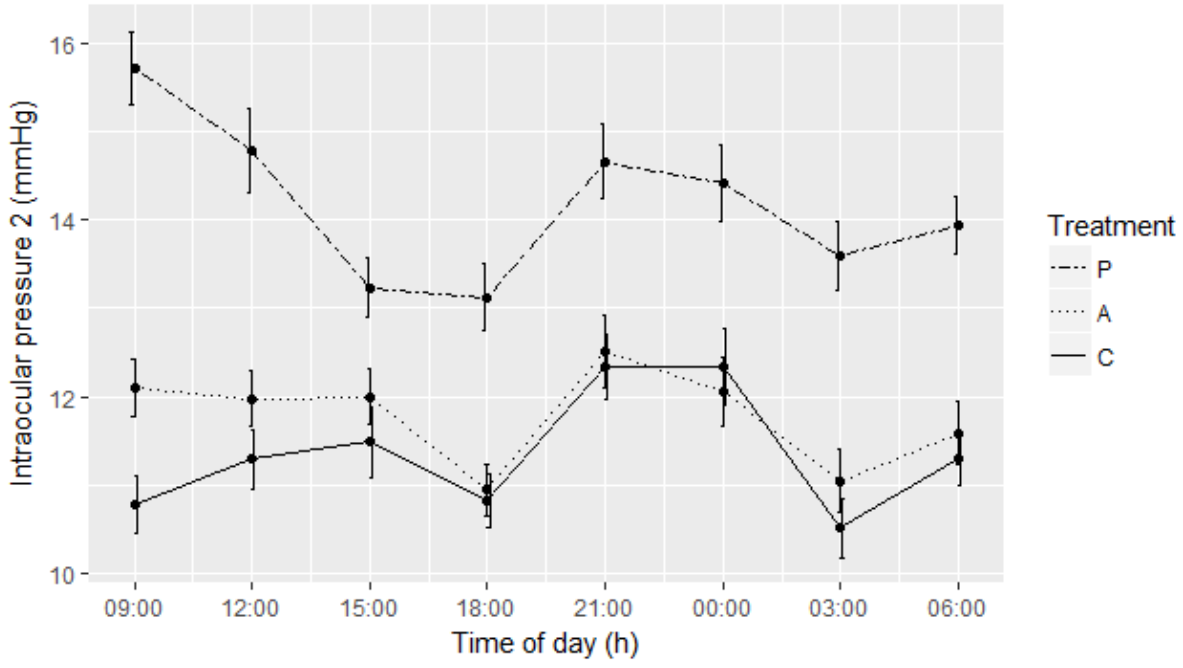


**Figure 6-** Mean intraocular pressure ($IOP_2$, in mmHg) of the treated eye during the day (in hours of the day) during treatment periods P, A, and C in ten healthy cats. P=placebo, A=dorzolamide 2% eye drops q8h, and C=dorzolamide 2% eye drops q8h + timolol 0.5% eye drops q12h. Standard errors of each IOP value were depicted in this figure with bars.

The mean PD values of the treated eye during treatment P, A and C were 5.1 ± 0.1 mm, 5.4 ± 0.1 mm and 4.3 ± 0.1 mm, respectively (Table 10). The moderation effect was significant which meant that light intensity had an significant effect on the PD (Table 9a-b). While taking into account the moderation effect of the light intensity on the pupil diameter, treatment C had a significant negative effect on the pupil diameter of the treated eye when compared to the treated eye during the placebo period ($p < 0.001$) (Table 9a). No significant difference between the PD of the untreated eye in treatment C when compared with the PD of the untreated eye during the placebo period was found ($p = 0.0547$) (Table 9b). Mean PD values of the untreated eye during treatment P, A and C were 5.1 ± 0.1 mm, 6.0 ± 0.1 mm and 6.1 ± 0.1 mm, respectively (Table 10). PD of the treated eyes during treatment A and C were both significantly smaller when compared to respectively the PD of the untreated eyes during treatment A and C ($p < 0.001$). There was no significant difference between the PD of the treated and untreated eye during the placebo period ($p = 0.995$).

**Table 9a-** Mean horizontal pupil diameter (PD) in mm and the effect of light intensity at eye level (LUX.BOOG) in lux of the treated eye of 10 healthy cats during six treatment periods. Intercept = PD in mm of the treated eye in the placebo period in which lux=0, LUX.BOOG=light intensity treated eye ,PT=PD in mm in the pre-treatment period, A=dorzolamide 2% eye drops q8h, B=brinzolamide 1% eye drops q8h, C=dorzolamide 2% eye drops q8h + timolol 0.5% eye drops q12h, and D=brinzolamide 1% eye drops q8h + timolol 0.5% eye drops q12h. Modl= lux*diameter. Estimate= the value of the PD in mm in which lux=0 and is shown as the difference of the treated eye when compared with the intercept of the placebo period. SE= standard error.

| Treatment | estimate | SE | p.ratio |
|---|---|---|---|
| (Intercept) | 4.576858 | 0.15569275 | 0.0000 |
| LUX.BOOG | -0.007646 | 0.00024485 | 0.0000 |
| PT | -0.034259 | 0.06449346 | 0.5953 |
| A | -0.205364 | 0.06583057 | 0.0018 |
| B | -0.035793 | 0.06502913 | 0.5821 |
| C | -1.421973 | 0.06656096 | 0.0000 |
| D | -1.252377 | 0.06614839 | 0.0000 |
| mod1 | 0.001996 | 0.00004873 | 0.0000 |

**Table 9b-** Mean horizontal pupil diameter (PD) in mm and the effect of light intensity at eye l evel (LUX.NBOOG) in lux of the untreated eye of 10 healthy cats during six treatment period s. Intercept = PD in mm of the untreated eye in the placebo period in which lux=0, LUX.NBO OG=light intensity untreated eye ,PT=PD in mm in the pre-treatment period, A=dorzolamide 2% eye drops q8h, B=brinzolamide 1% eye drops q8h, C=dorzolamide 2% eye drops q8h + ti molol 0.5% eye drops q12h, and D=brinzolamide 1% eye drops q8h + timolol 0.5% eye drops q12h. Modl= lux*diameter. Estimate= the value of the PD in mm in which lux=0 and is show n as the difference of the untreated eye when compared with the intercept of the placebo perio d. SE= standard error.

| Treatment | estimate | SE | p.ratio |
|---|---|---|---|
| (Intercept) | 4.618843 | 0.05717880 | 0.0000 |
| LUX.NBOOG | -0.015245 | 0.00016088 | 0.0000 |
| PT | -0.033667 | 0.03415210 | 0.3243 |
| A | 0.064516 | 0.03499377 | 0.0654 |
| B | 0.071831 | 0.03448743 | 0.0374 |
| C | 0.067874 | 0.03531602 | 0.0547 |

| | | | |
|---|---|---|---|
| D | 0.049153 | 0.03511643 | 0.1617 |
| mod1 | 0.003468 | 0.00002975 | 0.0000 |

As can be seen in Table 10, the mean HRs during treatment A and C were respectively 157 ± 1.1 bpm and 150 ± 1.0 bpm, an increase of 3.3% and a decrease of 1.3% when compared with the HR of 152 ± 1.2 bpm during the placebo period. There was no significant decrease in HR during treatment period C when compared with the HR in the placebo period ($p = 0.178$). The HR during treatment period A was significantly higher when compared to the HR during the p lacebo period ($p < 0.001$) and when compared to the HR during treatment C ($p < 0.001$).

**Table 10-** Mean intraocular pressure ($IOP_{1-2}$), pupil diameter (PD) and heartrate (HR) (± SE) and mean differences in $IOP_{1-2}$, PD and HR between treatment periods A,C and P. $IOP_{1,2}$ of the treated eye in mmHg, $IOP_2$* of the untreated eye in mmHg, PD of the treated eye in mm, PD* of the untreated eye in mm and HR in bpm. PT=pre-treatment period, of which mean values of day 3-5 were depicted in this table, P=placebo period, A=dorzolamide 2% eye drops q8h, and C=dorzolamide 2% eye drops q8h + timolol 0.5% eye drops q12h. P value indicated if differences between treatment periods were statistically significant ($p < 0.05$).

| Mean difference | | Values during period | | | | P value |
|---|---|---|---|---|---|---|
| parameter | comparison | A | C | P | PT | |
| **$IOP_1$** | | | | | | |
| | A-C | 12.5±0.1 | 12.0±0.1 | | | 0.011 |
| | A-P | 12.5±0.1 | | 14.9±0.2 | | < 0.001 |
| | C-P | | 12.0±0.1 | 14.9±0.2 | | < 0.001 |
| | | | | | 15.2±0.2 | |
| **$IOP_2$** | | | | | | |
| | A-C | 11.8±0.1 | 11.4±0.1 | | | 0.009* |
| | A-P | 11.8±0.1 | | 14.2±0.1 | | < 0.001* |
| | C-P | | 11.4±0.1 | 14.2±0.1 | | < 0.001* |
| **$IOP_2$*** | | | | | | |
| | A-C | 11.6±0.1 | 11.1±0.1 | | | 0.001* |
| | A-P | 11.6±0.1 | | 14.0±0.1 | | < 0.001* |
| | C-P | | 11.1±0.1 | 14.0±0.1 | | < 0.001* |
| **PD** | | | | | | |
| | A-C | 5.4±0.1 | 4.3±0.1 | | | |
| | A-P | 5.4±0.1 | | 5.1±0.1 | | |
| | C-P | | 4.3±0.1 | 5.1±0.1 | | |
| | | | | | 4.8±0.1 | |
| **PD*** | | | | | | |
| | A-C | 6.0±0.1 | 6.1±0.1 | | | |
| | A-P | 6.0±0.1 | | 5.1±0.1 | | |
| | C-P | | 6.1±0.1 | 5.1±0.1 | | |
| **HR** | | | | | | |
| | A-C | 157±1.1 | 150±1.0 | | | < 0.001* |
| | A-P | 157±1.1 | | 152±1.2 | | < 0.001* |
| | C-P | | 150±1.0 | 152±1.2 | | 0.178 |
| | | | | | 149±1.5 | |

**Discussion**

The objective of this study was to evaluate the effect of topical administration of dorzolamide 2% three times daily, either alone or in combination with topical administration of timolol 0.5% two times daily, on the intraocular pressure (IOP), pupil diameter (PD) and heart rate (HR) in healthy cats. In both treatment A (dorzolamide 2% eye drops q8h) and treatment C (dorzolamide 2% eye drops q8h + timolol 0.5% eye drops q12h) $IOP_2$ was significantly decreased when compared with baseline values from the placebo period ($p < 0.001$). However, $IOP_2$ was significantly more decreased during treatment C than during treatment A ($p = 0.009$). Treatment C had a significant negative effect on the pupil diameter related to lux of the treated eye when compared to the treated eye during the placebo period ($p < 0.001$). However, no significant difference between the PD related to lux of the untreated eye in treatment C when compared with the PD related to lux of the untreated eye during the placebo period was found ($p = 0.0547$). There was no significant decrease in HR during treatment period C when compared with the heartrate in the placebo period ($p = 0.178$).

To our knowledge, no studies have been performed to examine the IOP lowering effect of administering dorzolamide q8h in combination with administering timolol q12h in cats yet. The results of the present study were valuable since this medication combination has not yet been studied. Furthermore, our study included an acclimatisation period of 5 days and a placebo period of 5 days before the treatment periods of 5 days commenced. A study of Dietrich et al. also included a pre-treatment phase, however it took only two days and no acclimatisation to the eye drops was performed[8]. In the present study, $IOP_2$ values of day 1 and day 2 were significantly higher than $IOP_2$ values of day 4 and day 5 in the placebo period ($p < 0.05$) (Figure 3a-b and Table 4a-b), which suggests that cats experienced stress, or were at least aroused, during the first days of eye drop administration. This stresses the importance of an acclimatisation period and placebo period of sufficient days.

A habituation to IOP measurements was suspected in the present study since a significant ($p < 0.001$) decrease in $IOP_1$ in OS was measured as compared to the $IOP_1$ in OD (the eye in which IOP was always measured first). For this reason, the IOP was measured in two series per eye in a sequence of OD-OS-OD-OS from the placebo measurements onwards. Consequence was that no value of $IOP_2$ was available for the acclimatisation period. During the placebo period, the second measurement ($IOP_2$) had a significantly lower IOP value than $IOP_1$ ($p < 0.001$). In addition, $IOP_2$ values showed less dispersion when compared to $IOP_1$ values. For this reason it was likely that the recorded IOP in OS was lower than in OD due to a habituation or relaxation effect.

Other factors that may have influenced the measured IOP values were that the very first (09:00h) measurements of the placebo period were not performed due to circumstances. As a practical solution the lacking 09:00h data of day 1 were replaced by 09:00h measurements of day 2.

The measurements were performed mostly by the four students. However, incidentally it was not possible for them to perform the measurements, which were in this case taken over by the supervisors of the research project. The fact that different people performed the measurements may have led to a difference in measured values. However, according to a study of Görig et al. there were no significant differences in IOP measurements between experienced and inexperienced examiners when using a TonoVet tonometer.[12] Therefore it is not likely that the incidental measurements by the supervisors of the project influenced the IOP values.

During the medication periods the medication of 15:30h coincided with the measurement of 15:00h, which resulted in the medication being administered immediately after the measurements had been performed. This meant that cat number 11 received the medication

structurally up to an hour later than cat number 1. This difference in time of application could have resulted in differences in the IOP value measured afterwards. However, due to the fixed order in which medication was applied and measurements were performed, this potential error would occur consistently for all measurements in this specific cat.
Another factor which may have incidentally caused a false high IOP value was the fact that the examination room was located next to the dog kennels. Occasional sounds may have incited stress or arousal in the cats.
After the washout period of a medication period it was noticed that there still was an influence on the IOP of the last medication, due to the fact that after a treatment period which included timolol, a difference in PD between OD and OS was noticed. Comparing the baseline IOP value from the placebo period with the IOP value of the day before treatment, a significant ($p < 0.001$) decrease in IOP was observed which could be the result of the preceding treatment period. The effect of previous medication in combination with the short wash out period could have given a falsely lowered IOP value. In a study of Kiland et al. a washout period of three days was performed which was considered to be adequate[13]. For this reason the present study also used a washout period of three days.

The mean baseline $IOP_2$ (mean ± SD) in the treated eye, which was established during the placebo period, was 14.2 ± 0.1 mmHg. This was lower than the mean IOP (mean ± SD) of 19.7 ± 5.6 mmHg, which was reported in a previous study.[14] In that study, IOP was measured with an applanation tonometer (Tono-Pen). In the present study a rebound tonometer (Tono Vet) was used since in the lower and reference ranges of IOP, the Tono Vet provides readings much closer to the true, manometric IOP, than the Tono-Pen does.[15] In the present study SD values were also lower when compared to SD values of the study of Miller et al., which could have been the result of the fact that the second of two IOP measurements was used in the present study, which was lower than the first.[14] Another factor that may have been responsible for the lower IOP value was that the cats in the present study were all from the same age and breed and some of the cats were from the same litter, which meant that they were not representative of the wider population. The study of Miller et al. included a larger cat population which may have been more representative.[14] Other limitations of our cat population were the fact that two cats have fallen out during the research project, but ten cats proved to be sufficient for detecting significant differences, and that the cats suffered from the cat flu during the research project. However, clinical signs of the cat flu were mild and did not include any eye problems and besides that, possible effects of the cat flu were spread over all medication protocols by using the Latin Square design. Besides, a heart murmur was diagnosed in one of the research cats at the first day of the research project. The cat was examined by a Board-certified veterinary cardiologist, who did not detect clinical signs of heart disease but found mitral valve insufficiency and concurrent left ventricular hypertrophy on ultrasonographic examination of the heart. An increased heart rate was not detected, nor expected.

In our study, IOP showed significant 24-h variations in a circadian pattern. A circadian rhythm was previously reported in cats, with highest IOP values between 21:00h and 00:00h which corresponds to the dark period in which cats are usually active[3]. However, $IOP_2$ fluctuations which were found in the present study had a different pattern: the $IOP_2$ value during the placebo period at 09:00h was significantly higher than at 15:00h, 18:00h, 03:00h and 06:00h and the $IOP_2$ value at 12:00h was significantly higher than at 18:00h.The IOP fluctuations of the present study were not similar to the pattern which was found in the study of Del Sole et al.[3] The study of Del Sole et al. suggested that for each species, higher values

of IOP correlate with the awakening or activity phase. It can be hypothesized that the different circadian pattern of IOP found in our study may be explained by different housing conditions and activity patterns in our research cats as compared to the cats of Del Sole's study. The drop in IOP during the day relative to the observations during the night could only be appreciated since IOP values were also assessed during night-time.

The $IOP_2$ percentage drop at each time point of treatment C compared with the placebo period: with 4.9 mmHg (31.2%) at 09:00h, with 3.4 mmHg (23.1%) at 12:00h, with 1.7 mmHg (12.9%) at 15:00h, with 2.3 mmHg (17.6%) at 18:00h, with 1.9 mmHg (13.2%) at 21:00h, with 1.4 mmHg (10.1%) at 00:00h, with 3.2 mmHg (23.5%) at 03:00h and with 2.6 mmHg (18.7%) at 06:00h. These results suggest the presence of a greater IOP-decreasing effect at time points at which the baseline value of IOP is high. Besides the IOP assessments were PD and HR also obtained. No significant decrease in HR in treatment C was found when compared with the HR in the placebo period. A decrease in HR was expected since the administration of timolol caused a significant HR decrease in a study of Gunther-Harrington et al.[16] However the study of Gunther-Harrington et al. did not include an acclimatisation period. Bearing in mind the decrease in IOP over the first two days of the adjustment period in our study, we hypothesize that the decrease in HR in the study by Gunther-Harrington et al. might at least in part be attributable to adjustment of the cats to the measurements, rather than to a systemic effect of the topically applied timolol eye drops.

A significant decrease in HR was found when the HR during treatment C was compared with the HR during treatment A ($p < 0.001$). Similar to a previous study, a significant decrease in PD was found in the treated eye during treatment C when compared with the treated eye during the placebo period ($p < 0.001$).[13] The PD in the untreated eye during treatment C did not differ significantly from the PD of the untreated eye in the placebo period ($p = 0.0547$). A decrease in PD in the untreated eye has been previously reported and it was hypothesized that timolol may enter the general circulation[7]. However, this effect did not occur in the present study. Miosis which occurred in the treated eye, was likely caused by beta-adrenergic inhibition or by alpha-adrenergic activation of the muscle of the iris sphincter.[11]

The mean $IOP_2$ values of the treated eye during treatment A and C were respectively $11.8 \pm 0.1$ mmHg and $11.4 \pm 0.1$ mmHg. The mean overall decrease of $IOP_2$ in treatment A and C were 2.4 mmHg (16.9%) and 2.8 mmHg (19.7%), respectively. In both treatments, a significant decrease in $IOP_2$ was observed when compared with their baseline values from the placebo period ($p < 0.001$). $IOP_2$ of treatment C was significantly lower than $IOP_2$ of treatment A ($p = 0.009$). To our knowledge, no studies have been performed to examine the IOP lowering effect of administering dorzolamide q8h in combination with administering timolol q12h in cats. However, Dietrich et al. examined the effect of administering dorzolamide q12h in combination with timolol q12h on the IOP in cats. The study of Dietrich et al. did not report a significantly greater IOP lowering effect of dorzolamide q12h in combination with timolol q12h than the IOP lowering effect of administering dorzolamide q8h alone.[8] The percentage decrease in mean overall $IOP_2$ in treatment A was slightly higher than the 12.1% decrease in IOP in the study of Dietrich et al. in which dorzolamide was applied three times daily.[8] These different results may have been the result of the differences in study design. For example, Dietrich et al. used an applanation tonometer (Tono-Pen), and control cats to which the treated cats were compared.[8] However, in the present study we compared the treated eyes of cats with the baseline of the treated eyes of the same cats.

A study similar to that of Dietrich et al. has been performed in dogs, in which the combination of dorzolamide and timolol two times daily showed a significant decrease in IOP when compared with the treatment of dorzolamide three times daily alone.[17] In humans, the IOP lowering effect of administering dorzolamide + timolol three times daily was significantly

greater than the IOP lowering effect of dorzolamide + timolol administered two times daily.[18] The results of the present study suggest that the topical administration of dorzolamide 2% three times daily in combination with timolol 0.5% two times daily has a significantly greater IOP decreasing effect than the administration of topical dorzolamide 2% three times daily alone. However, the difference in IOP during treatment A and C was not large. And since the cats in the present study did not suffer from glaucoma, research in glaucoma cats is needed to determine the clinical relevance of the (statistically) significantly greater IOP decreasing effect of administering of dorzolamide 2% three times daily in combination with timolol 0.5% two times daily.

The results presented in this study show that since the highest IOP drop of the combination therapy in this research was observed when the IOP value was at its highest (09:00h), it can be expected that the combination therapy will have an increased IOP decreasing effect treatment potential in glaucoma cats suffering from high IOP values.

## Conclusion

The results of the present study show that in healthy cats the topical administration of dorzolamide 2% TID in combination with timolol 0.5% BID has a significantly greater IOP lowering effect than the administration of dorzolamide 2% TID alone. A study on the effect of the combined administration of dorzolamide and timolol in cats with glaucoma is warranted in order to determine the clinical relevance of the findings in the present study.

## Acknowledgement

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## References


1. Maggio, F. Glaucomas. *Top. companion Anim. Med.* **30,** 86–96 (2015).
2. Ruiz, R. S., Wilson, C. A., Musgrove, K. H. & Prager, T. C. Management of increased intraocular pressure after cataract extraction. *Am. J. Ophthalmol.* **103,** 487–491 (1987).
3. Del Sole, M. J., Sande, P. H., Bernades, J. M., Aba, M. A. & Rosenstein, R. E. Circadian rhythm of intraocular pressure in cats. *Vet. Ophthalmol.* **10,** 155–161 (2007).
4. Shahidullah, M., Wilson, W. S., Yap, M. & To, C. Effects of ion transport and channel-blocking drugs on aqueous humor formation in isolated bovine eye. *Invest. Ophthalmol. Vis. Sci.* **44,** 1185–1191 (2003).
5. Maren, T. H. The rates of movement of Na+, Cl-, and HCO-3 from plasma to posterior chamber: effect of acetazolamide and relation to the treatment of glaucoma. *Invest. Ophthalmol.* **15,** 356–364 (1976).
6. McLellan, G. J. & Miller, P. E. Feline glaucoma – A comprehensive review. *Vet. Ophthalmol.* **14,** 15–29 (2011).
7. Frishman, W. H., Fuksbrumer, M. S. & Tannenbaum, M. Topical ophthalmic beta-adrenergic blockade for the treatment of glaucoma and ocular hypertension. *J. Clin. Pharmacol.* **34,** 795–803 (1994).
8. Dietrich, U. M., Chandler, M. J., Cooper, T., Vidyashankar, A. & Chen, G. Effects of topical 2% dorzolamide hydrochloride alone and in combination with 0.5% timolol maleate on intraocular pressure in normal feline eyes. *Vet. Ophthalmol.* **10 Suppl 1,** 95–100 (2007).
9. Rainbow, M. E. & Dziezyc, J. Effects of twice daily application of 2% dorzolamide on intraocular pressure in normal cats. *Vet. Ophthalmol.* **6,** 147–150 (2003).
10. Rankin, A. J., Crumley, W. R. & Allbaugh, R. A. Effects of ocular administration of ophthalmic 2% dorzolamide hydrochloride solution on aqueous humor flow rate and intraocular pressure in clinically normal cats. *Am. J. Vet. Res.* **73,** 1074–1078 (2012).
11. Wilkie, D. A. & Latimer, C. A. Effects of topical administration of timolol maleate on intraocular pressure and pupil size in cats. *Am. J. Vet. Res.* **52,** 436–440 (1991).
12. Görig, C., Coenen, R. T. I., Stades, F. C., Djajadiningrat-Laanen, S. C. & Boevé, M. H. Comparison of the use of new handheld tonometers and established applanation tonometers in dogs. *Am. J. Vet. Res.* **67,** 134–144 (2006).

13. Kiland, J. A., Voss, A. M. & McLellan, G. J. Effect of timolol maleate gel forming solution on intraocular pressure, pupil diameter, and heart rate in normal and glaucomatous cats. *Vet. Ophthalmol.* **19,** 91–96 (2016).
14. Miller, P. E., Pickett, J. P., Majors, L. J. & Kurzman, I. D. Evaluation of two applanation tonometers in cats. *Am. J. Vet. Res.* **52,** 1917–1921 (1991).
15. McLellan, G. J., Kemmerling, J. P. & Kiland, J. A. Validation of the TonoVet® rebound tonometer in normal and glaucomatous cats. *Vet. Ophthalmol.* **16,** 111–118 (2013).
16. Gunther-Harrington, C. T., Ontiveros, E. S., Hodge, T. E., Visser, L. C. & Stern, J. A. Effects of 0.5% timolol maleate ophthalmic solution on heart rate and selected echocardiographic indices in apparently healthy cats. *J. Vet. Intern. Med.* **30,** 733–740 (2016).
17. Plummer, C. E., MacKay, E. O. & Gelatt, K. N. Comparison of the effects of topical administration of a fixed combination of dorzolamide–timolol to monotherapy with timolol or dorzolamide on IOP, pupil size, and heart rate in glaucomatous dogs. *Vet. Ophthalmol.* **9,** 245–249 (2006).
18. Shemesh, G., Moisseiev, E., Lazar, M. & Kurtz, S. Intraocular pressure reduction of fixed combination timolol maleate 0.5% and dorzolamide 2% (Cosopt) administered three times a day. *Clin. Ophthalmol. Auckl. NZ* **6,** 283–287 (2012).