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**Short-term effects of ocular 2% dorzolamide, 0.5% timolol or
0.005% latanoprost on the anterior segment architecture in
healthy cats: a prospective study.**

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23 **Abstract**

24 Dorzolamide 2%, timolol 0.5% and latanoprost 0.005% are widely used in the medical
25 management of glaucoma in humans and pets. In this study, we wanted to evaluate the effect
26 of these three molecules belonging to different hypotensive families on intraocular pressure
27 (IOP) and anterior segment architecture in clinically healthy cats and compare these data with
28 those obtained in a previous study under the same conditions in healthy dogs. In this prospective
29 study 45 cats were included and divided into 3 homogeneous groups. For 7 days eye drops were
30 instilled in the right eye (treated eye), dorzolamide 2% BID for the first group, timolol 0.5%
31 BID for the second group and latanoprost 0.005% SID for the third group. The left (untreated)
32 eye of all these cats constitute a control group useful for the statistical analysis of the data. On
33 D0 and D7, the IOP was measured with Tonovet® and then, a high frequency ultrasound
34 (ultrasound biomicroscopy UBM) was undertaken under general anesthesia. A biostatistical
35 study of the 1440 data was then performed. Statistical test validated the use of the controlateral
36 eye as a control, and the representativeness of a sample of 15 animals per group. At D7, the
37 IOP of healthy cats treated with dorzolamide 2% BID decreased significantly whereas no
38 variation was obtained with instillation of timolol 0.5% and latanoprost 0.005%. According to
39 our protocol, these three hypotensive molecules do not significantly affect the architecture of
40 the anterior segment of the clinically healthy feline eye as in dogs.

41

42 **Keywords**

43 Cats, dorzolamide, latanoprost, timolol, UBM.

44

45 **Running title**

46 Effects of three anti-glaucomatous drugs on the anterior segment architecture in cats.

47

48 **Introduction**

49 A common feature of all glaucoma in animals is the neurodegenerative disorder of retinal
50 ganglion cells (RGCs) and their axons, an abnormal increase in intraocular pressure (IOP)
51 represents a constant risk factor [Stiles, 2013]. The IOP is maintained at a relatively constant
52 level and the rate of aqueous humor (AH) formation equals the outflow. Increase in IOP results
53 from impairment of the aqueous humor outflow through the trabecular conventional pathway
54 and/or through the uveoscleral pathway. It is estimated that glaucoma can affect about 1% of
55 cats over the age of seven [Kroll et al., 2001] and most cases of feline glaucoma are secondary
56 [Stiles, 2013].

57 Current therapy for feline glaucoma follows the same guidelines used in other species. Medical
58 therapeutic option includes many topical hypotensive agents used in humans and dogs. Among
59 hypotensive drugs, 3 families are commonly used: carbonic anhydrase inhibitors (such as
60 dorzolamide), beta-blockers (such as timolol) and prostaglandin analogs (such as latanoprost).
61 Dorzolamide reduces the active formation of HA by inhibiting the action of carbonic anhydrase,
62 which is found abundantly in the ciliary body epithelial cells. Timolol as a beta-blocker, lowers
63 aqueous humor flow by altering the adrenergic neuronal control of aqueous humor formation
64 by blockade of the beta-receptors in the ciliary body processes, it may also inhibit active
65 transport and ultrafiltration related to sodium transfer system. Latanoprost reduces IOP by
66 increasing the uveoscleral outflow. The remodeling of the extracellular matrix between the
67 ciliary muscle fibers by local metalloproteases contributes to this pharmacological effect
68 [Plummer et al., 2013].

69 In a recent study by Poinard et al., 2018, these three hypotensive drugs lowered significantly
70 IOP in dogs without modification of the biometry of the iridocorneal angle (ICA). This result
71 is consistent with the hypotensive effect of dorzolamide and timolol on ciliary body epithelium
72 but it is more surprising with latanoprost which increases the uveoscleral outflow of aqueous

73 humor, all the more so as a previous study shows that the biometry of the anterior segment and
74 the ciliary cleft is modified two hours after the application of a drop of latanoprost 0,005% in
75 dogs [Gelatt and Mackay, 2001].

76 The goal of this study was to compare the effect of these three hypotensive molecules on IOP
77 and biometry of the anterior segment of healthy cats, as well as to compare these data with
78 those obtained by Poinsard et al., 2018 in healthy dogs with the same protocol under the same
79 conditions. To evaluate the biometry of the anterior segment, we used high-frequency
80 immersion ultrasound with high definition (20-50µm) for structures up to 5mm deep
81 [Leibmann, 1998], while being non-invasive, and for which most of the parameters of the
82 anterior segment have already been objectified in healthy cats [Aubin, 2003].

83

84 **Materials and Methods**

85

86 1. Inclusion criteria

87 This prospective study with a control group was performed using 45 healthy cats without any
88 current local or systemic treatment. These 45 cats belonged to staff or customers of the
89 Lorrainevet veterinary clinic. All the dogs were included with the consent of their owners. A
90 complete general and ophthalmic examination including slit-lamp biomicroscopy and indirect
91 ophthalmoscopy without pupillary dilatation was performed, followed by an evaluation of IOP
92 using a rebound tonometer (Tonovet®, Icare, Vantaa, Finland). All animals with an
93 ophthalmologic history or a difference greater than 2 mmHg IOP (on an average of three IOP
94 measurements) on both eyes were excluded from the study.

95

96 2. Experimental protocol

Commenté [MOU1]: cats

97 Cats were divided into 3 homogeneous groups (weight, age, sex, race) of 15 cats (Table I). The
98 study was done in two steps, spaced 7 days apart. The first step (D0) was performed in a
99 mesopic environment, a complete general and ophthalmic examination, without dilation and
100 without any instillation, was carried out between 10 am and 1 pm for each animal. IOP was an
101 average of three measures on each eye. Ultrasound biomicroscopy (UBM) examination
102 (Aviso®, Quantel Medical, Cournon d'Auvergne, France) was then performed under isoflurane
103 gas anesthesia after medetomidine premedication and ketamine induction. At the awakening of
104 each animal, eye drops were instilled into the right eye. For group 1, one drop of dorzolamide
105 2% (Trusopt 20mg / ml eye drops ND, MSD, Courbevoie, France) was instilled twice daily, at
106 7am and 7pm, from D0 at 7pm until D7 at 7am. For group 2, one drop of 0.5% timolol
107 (Timoptol 0.5% eye drops, MSD, Courbevoie, France) was instilled twice daily according to
108 the same protocol as group 1. For group 3, one drop of latanoprost 0.005% (Xalatan 0.005%
109 eye drops ND, Pfizer, Paris, France) was instilled once a day at 10pm in the fornix of the right
110 eye for seven days, from D0 at 10 pm until D6 at 10 pm included.

111 In the second step, on day 7, all animals were re-examined 12 hours after the last drop of
112 latanoprost and 6 hours after the last drop of dorzolamide or timolol, with the same procedure
113 under the same conditions as on D0. All examinations and UBM ultrasound were performed by
114 the same operator on D0 and D7.

115 3. UBM high frequency ocular ultrasound

116 The ultrasound biomicroscopy for this study was a 50Mhz monotrducer with immersion
117 probe with a geometric focalization and line scanning (UBM Aviso®, Quantel Medical,
118 Cournon d'Auvergne, France). The patient was positioned in dorsal recumbency with its head
119 stabilized in a vacuum pillow. Two video sequences were recorded for each eye at D0 and D7.
120 The first sequence was recorded by placing the probe both centrally and perpendicular to the
121 corneal surface to determine the distance between the apex of the corneal endothelium and the

122 apex of the anterior capsule of the lens, at the 12 o'clock position. In the second sequence, the
123 outflow pathways were evaluated in the dorsal quadrant with the probe placed perpendicular to
124 the corneoscleral limbus at the 12 o'clock position. From each video sequence, one image was
125 chosen for analysis (Figure 1).

126 4. The anterior segment parameters

127 Nine parameters were evaluated (Figure 2): (a) the ICA , (b) the width of the entrance of the
128 ciliary cleft (CC), (c) the width of the mid-CC, (d) the length of CC, (e) the depth of the anterior
129 chamber (AC), (f) the thickness of cornea at the corneoscleral limbus note, (g) the distance
130 between Schwalbe's line (the borderline between the cornea and sclera) and the anterior lens
131 capsule according to Kawata and Hasegawa., 2013 and (h) the area of the ciliary cleft. These
132 parameters were those described by Dulaurent et al., 2012 and used by Poinard et al., 2018.
133 The ICA (a) was the angle formed by the base of the iris in the region of the CC entry and the
134 inner corneoscleral junction. The length of the CC noted (d) corresponded to the distance
135 between the pectinate ligament (or the most anterior visible portion of the uveal trabecular
136 meshwork) and the anterior part of the ciliary body. The width of the CC entry, marked (b) was
137 the distance between the corneoscleral limbus and the iris root. The width of the mid-CC (c)
138 was the distance between the inner sclera and the ciliary process in the central portion of the
139 CC. The anterior chamber depth (e) was determined as the distance between the corneal
140 endothelium in the apical region of the cornea and the anterior pole of the lens. The thickness
141 of the cornea at the limbus (f) was the measure between the epithelium of the cornea and the
142 endothelium in its most peripheral transparent part. The distance between the anterior capsule
143 of the lens and the Schwalbe's line (g) was described by Kawata and Hasegawa., 2013. The
144 area of the ciliary cleft (h) was calculated by the area formula $(CC) = (d / 2 \times (b + c) / 2) +$
145 $((cxd) / 4)$. All these measurements were performed by the UBM software caliper.

146 5. Statistical evaluation

147 For this study, 45 cats were divided into 3 homogeneous groups (weight, racial type, sex, age)
148 of 15 individuals. Sample size $n = 15$ was determined using Student's t-test. Only five breeds
149 of cats with a majority of European cats was represented in the three groups, the distribution
150 was made in such a way as to obtain ages between 1 year and 10 years, an average weight of
151 4.1 kg and a male / female ratio close to 50% (Table I). Each animal was submitted to only one
152 experiment (one type of eye drops) and two general anesthesia 7 days apart. The 1440 data
153 obtained, including measurements of IOP and anterior segment biometry on the left (untreated)
154 and right (treated) eyes, on D0 and D7 were integrated into a statistical model, in which the
155 experimental unit was a cat, and the variable factor was the type of eyedrops instilled for 7 days.
156 The objective of the statistical study was to determine the effects of these three molecules on
157 anterior segment biometry and IOP. For this, two statistical methods were used: the non-
158 parametric Wilcoxon test to evaluate the significant or non-significant differences between two
159 independent groups and a one-way analysis of variance (ANOVA) to compare the sample
160 means. The level of significance was set at $p < .05$ for all statistical analyses.

161

162 **Results**

163

164 The IOP and biometric measurements of the anterior segment of the untreated eye (left eye) did
165 not significantly differ between D0 and D7. And no significant difference in the biometric data
166 of the anterior segment was found on day 0 between the untreated eye and the treated eye. After
167 one week of instillation of dorzolamide 2%, the IOP of the treated eye was significantly 1mmHg
168 lower (Figure 3). This decrease was relatively moderate (5.3%). After one week of instillation
169 of latanosprost or timolol, the IOP of the treated eye did not significantly decrease (Figure 3).
170 About biometric data, ICA values (a), the width of the entrance of the CC (b), the width of the
171 mid-CC (c), the length of the CC (d), the anterior chamber depth (e), the distance between the

172 anterior capsule of the lens and the Schwalbe's line (g) and the area of the ciliary cleft (h) were
173 not significantly altered after 7 days of instillation whatever the eye drops. Only a significant
174 decrease of 1.4% in corneal thickness (f) was observed with the instillation of dorzolamide
175 (Figure 4), whereas there was a significant increase of 1.6% in thickness of the cornea (f) with
176 the instillation of timolol (Figure 4).

177

178 **Discussion**

179

180 In this study, the parameters measured on the untreated eye (IOP and biometric data) did not
181 vary between D0 and D7. It was therefore admitted that eye drops instilled on the treated eye
182 (OD) for 7 days had no significant effect on the parameters measured on the untreated eye (OS)
183 and this despite a possible systemic diffusion of eye drops. In addition, the absence of a
184 significant difference in the data measured between the treated (OD) and untreated (OS) eye on
185 D0 allowed to validate the use of the untreated eye (OS) as a control at D0 and D7. The study
186 could also have been performed by comparing the data of the treated eye (OD) to D0 and D7.
187 Both analyses were performed with the same statistical tests and we observed the same
188 variations.

189 In contrast with healthy dogs for which these three types of hypotensive agents cause a
190 significant decrease in IOP [Poinsard et al., 2018], only instillation of dorzolamide at 2% twice
191 daily for 7 days resulted in a moderate but significant decrease in IOP in healthy cats in this
192 study. This result is similar to previous studies evaluating the effects of 2% dorzolamide
193 instilled in the eyes of healthy cats [Rainbow and Dziezyc, 2003], [Dietrich et al., 2007] and
194 [Rankin et al., 2012]. This moderate decrease in IOP of 1 mmHg (5.5%) is close to the decrease
195 (1.6 mmHg) in the Dietrich study (2007) when 2% dorzolamide is instilled twice daily.
196 Increasing the frequency of instillation of dorzolamide 2% to 3 times daily does not appear to

197 significantly increase the hypotensive effect of dorzolamide in healthy cats [Dietrich et al.,
198 2007]. This modest effect of topical carbonic anhydrase inhibitors on the feline eye is also
199 viewed in an earlier study of 20 healthy cats, where IOP did not significantly decrease after
200 instillation of 1% brinzolamide for 7 days, twice a day [Gray and al., 2003]. This moderate
201 response in healthy cats does not question the interest of dorzolamide in the medical
202 management of glaucoma in cats, since the Sigle study in 2011 [Sigle and al., 2011] reveals an
203 interesting decrease in IOP of 7 cats with primary congenital glaucoma with dorzolamide
204 instilled 3 times a day.

205 The instillation twice daily of timolol for 7 days on the eyes of healthy cats did not lead to a
206 significant reduction in IOP between day 0 and day 7 in this study. This result is similar to that
207 obtained in the Kiland study [Kiland and al., 2016] and contrary to the older studies of Wilkie
208 and Latimer in 1991 and Colasanti and Trotter in 1981. In the Dietrich study (2007),
209 concomitant administration of dorzolamide and timolol did not have a more intense hypotensive
210 effect than dorzolamide alone.

211 Finally, instillation of latanoprost once daily for 7 days in healthy cats' eyes did not decrease
212 the IOP between day 0 and day 7 in this study, which is consistent with previous studies on the
213 effects of latanoprost on the IOP of healthy cats [Mc Donald and al., 2016], [Studer and al.,
214 2000]. This lack of efficacy of PGF2 alpha receptor analogs is attributed to species differences
215 in prostaglandin receptors in the ciliary bodies of the cat's eye that express EP and DP receptors
216 rather than FP receptors, found in particular in humans [Regnier and al., 2006].

217 The absence of significant modification of the anterior segment biometry in this study during
218 instillation of timolol and latanoprost in healthy cats confirms the lack of response of these
219 molecules to IOP after 7 days of treatment.

220 Regarding dorzolamide 2%, the absence of significant modification on the biometry of the
221 anterior segment confirms that dorzolamide acts on the IOP by lowering the production of HA

222 without interacting on the outflow pathway of HA. The lack of effect on AIC biometrics of
223 these three molecules in healthy cats is similar to the results obtained in healthy dogs using the
224 same protocol and the same operator [Poinsard and al., 2018]. A decrease of 1.4% in the
225 thickness of the peripheral cornea in cats treated with dorzolamide was found, this decrease
226 similar to treated dogs [Poinsard and al., 2018] can be related to a transient dehydration of the
227 cornea. Conversely, a 1.6% increase in the thickness of the peripheral cornea in cats treated
228 with timolol was noted: this can be related to a transient edema of the cornea.

229 Differences in IOP results with some studies during instillation of timolol may be related to the
230 type of tonometer used. Nevertheless, Tonovet® provides accurate and reproducible IOP
231 measurements in cats [McLellan and al., 2013]. Although all the qualitative measurements on
232 the anterior chamber angle and sclerociliary cleft are perfectly described by Dulaurent et al.,
233 2012, the precision of the UBM measurements remains a variable dependent on the experience
234 of the operator. It is important to remember that the comparison of the data between healthy
235 dogs and healthy cats is reinforced by the fact that these two studies were carried out by the
236 same operator and under the same conditions.

237 This study has the same limitations as the study by Poinsard et al., 2018, limitations related to
238 the recruitment mode (private structure) causing a double selection bias, especially for breeds
239 that may have specificities, and the number of cats presented. Even if these three molecules
240 showed little or no influence on IOP and anterior segment biometry in healthy cats, it would be
241 interesting to carry out the same study in a context of glaucomatous cats.

242

243 **Conclusion**

244 This prospective study revealed a significant decrease in IOP in healthy cats treated with
245 dorzolamide at 2% BID for 7 days. Timolol 1% BID and latanoprost SID have no effect on IOP
246 after 7 days of treatment in healthy cats. Finally, dorzolamide 2%, timolol 0.5% and latanoprost

247 0.005% have no effect on the biometry of AIC in healthy cats with the protocol used and the
248 duration studied.

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254 Bastogne for the statistical analysis.

255

256 **Conflict of interest:**

257 The Author(s) declare(s) that there is no conflict of interest.

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325

326 **Iconography**

327

328 Figure 1: Ultrasound biomicroscopy (UBM) of the iridocorneal angle and ciliary cleft in a
 329 normal cat.

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331 Figure 2: Histological aspect of the anterior segment of a dog's eye showing the main biometric
 332 parameters studied in cats.

333

334 Table I: Summary of race, age, gender and weight data for cats in the cohort.

335

336 Figure 3: Box chart of IOP variations after one week of anti-glaucoma therapy on the treated
 337 eye (DOR: dorzolamide 2%, TI: timolol 0.5%, LAN: latanoprost 0.005%).

338

339 Figure 4: Box chart of corneal thickness variations (f) after one week of anti-glaucoma
 340 treatment on the treated eye.

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342 Table II: Mean value of the IOP (mmHg) after one week of anti-glaucoma treatment on the
 343 treated eye.

344

345 Table III: Mean value of the corneal thickness (f) (mm) after one week of anti-glaucoma
 346 treatment on the treated eye.

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348 Table IV: Mean value of the width of the entrance of the ciliary cleft (b) (mm) after one week
 349 of anti-glaucoma treatment on the treated eye.

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354 Table I: Summary of race, age, gender, and weight data for cats in the cohort

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Group 1				Group 2				Group 3			
Breed	Age	Gender	Weight	Breed	Age	Gender	Weight	Breed	Age	Gender	Weight
European	5	Mn	4	European	8	Fn	5	European	1	Mn	4
European	2	Fn	4	European	1	Mn	3	European	1	Fn	3
European	8	Mn	5	European	1	Mn	3	Persian	1	M	4
European	1	Fn	4	European	6	Fn	5	European	7	Mn	5
European	1	Fn	6	European	1	Mn	3	European	6	Fn	6
Persian	2	Mn	3	European	8	Mn	6	European	9	Fn	5

European	1	F	3	Oriental shorthair	1	Mn	3	European	4	Mn	6
European	4	Mn	4	European	2	Fn	4	European	4	Mn	4
European	6	Mn	4	European	6	Fn	5	European	6	Fn	4
European	1	Mn	6	European	10	Mn	5	European	1	Mn	3
European	8	Fn	3	European	8	Fn	3	European	4	Fn	4
European	9	Mn	6	European	3	Mn	4	European	2	Mn	3
European	6	Fn	3	European	3	Fn	4	European	8	Mn	5
Somali	4	F	2	Abyssin	2	Fn	3	European	4	Mn	4
European	10	Fn	5	European	1	Fn	3	Somali	1	Fn	3

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365 **Table II:** Mean value of the IOP (mmHg) after one week of anti-glaucoma treatment on the
366 treated eye.

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	Dorzolamide 2%	Timolol 0,5%	Latanoprost 0,005%
PIO Mean D0 (mmHg)	17,6	16,8	18,3
PIO Mean D7 (mmHg)	16,6	17	18,6

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Table III: Mean value of the corneal thickness (f) (mm) after one week of anti-glaucoma treatment on the treated eye.

	Dorzolamide 2%	Timolol 0,5%	Latanoprost 0,005%
(f) Mean D0 (mm)	0,64	0,63	0,64
(f) Mean D7 (mm)	0,63	0,64	0,64

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418 Table IV: Mean value of the width of the entrance of the ciliary cleft (b) (mm) after one week
419 of anti-glaucoma treatment on the treated eye.
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	Dorzolamide 2%	Timolol 0,5%	Latanoprost 0,005%
(b) Mean D0 (mm)	0,82	0,80	0,85
(b) Mean D7 (mm)	0,84	0,8	0,86

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