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ORIGINAL REPORT

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Increased drug concentration and repeated eye drop administration as strategies to optimize topical drug delivery: A fluorophotometric study in healthy dogs

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Abstract

Objectives: Determine tear film kinetics with different fluorescein concentrations and repeated eye drop administration at various time intervals.

Animals Studied: Six healthy Beagles.

Procedures: Six experiments were conducted on separate days: single eye drop administration (control) or two separate eye drops administered at 30 s, 1, 2, 5, and 10 min intervals. For each experiment, one eye received 0.3% fluorescein solution while the other eye received 1% fluorescein solution, and tear fluid was collected with capillary tubes at 0, 1, 5, 10, 20, 30, 40, 50, 60, 90, 120, and 180 min. Fluorescein concentrations were measured using automated fluorophotometry.

Results: Compared with 0.3% solution, eyes receiving 1% fluorescein solution had significantly higher tear film concentrations ($p \leq .046$) and the area-under-the-fluorescein-time curve was twofold greater ($p = .005$). Compared with control: (i) Tear film concentrations were significantly higher for up to 20 min when repeating administration 30 s to 5 min after the first drop ($p \leq .006$); (ii) The highest increase in area-under-the-curve was obtained with 2 and 5 min intervals for 0.3% (+109%–130%) and 1% solutions (+153%–157%); (iii) The highest increase in median precorneal retention time (defined as tear film concentration < 5% from baseline values) was obtained with 5 min intervals for 0.3% (55 min vs. 15 min in control) and 2–5 min intervals for 1% solutions (50 min vs. 25 min in control).

Conclusions: Drug delivery to the ocular surface can be enhanced by using more concentrated formulations and/or by repeating eye drop administration 2–5 min after the first dose.

KEYWORDS

canine, drug delivery, eye drop, pharmacokinetics, precorneal retention, tear film

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1 | INTRODUCTION

Ophthalmic solutions ("eye drops") represent the main formulations for treating ocular conditions in veterinary and human patients. Eye drops provide a convenient, simple, and non-invasive route of administration that achieves relatively high drug concentrations at the target tissue(s) while minimizing the risk for systemic drug toxicity.[1] However, the ocular bioavailability of eye drops is generally poor owing to rapid precorneal loss from reflex blinking and efficient nasolacrimal drainage.[2–4] In canine eyes, tear film concentrations rapidly decrease by ~20% and ~45% at 1 min post-administration of an ophthalmic solution (fluorescein) or ophthalmic suspension (prednisolone acetate), respectively.[5,6] As such, there is a critical need to optimize eye drop delivery to improve therapeutic benefits for clinical patients.

One could consider repeating the administration of the same eye drop to improve drug concentrations in the eye. On the one hand, if the second eye drop is applied at the same time as the first eye drop (i.e., instillation of two drops instead of one), drug concentrations are higher immediately after topical delivery (due to lower dilution effect from tears), but the pharmacokinetic benefits are short-lived (<1 min) and not clinically important.[5] In fact, the canine ocular surface can only "hold" the volume of one drop at a time (~35 μL); therefore, the second drop is mostly "wasted" from a pharmacological standpoint due to spillover on the periocular skin and rapid drainage through the nasolacrimal duct.[5] On the other hand, it is plausible that a second eye drop instilled after a certain lag period would be advantageous, but to the authors' knowledge, it has not been studied to date. Drug concentrations in the eye could also be enhanced by increasing drug concentrations in the ophthalmic formulations. In humans, Holland et al.[7] showed significantly greater drug concentrations in the cornea (ninefold) and aqueous humor (18-fold) following topical administration of 1.5% levofloxacin versus 0.3% gatifloxacin, while Bucci et al.[8] showed significantly greater aqueous humor concentrations and overall drug exposure (area-under-the-curve) with 1.5% levofloxacin compared with 0.5% moxifloxacin. In both cases, the higher drug concentration of the levofloxacin formulation (1.5% vs. 0.3%–0.5%) played an important role in explaining the study results. The same may be true in dogs.

The present study evaluated two modalities that could be used to improve ocular drug delivery when using eye drops in dogs, that is, repeated administration at various time intervals and increased drug concentration in the ophthalmic formulation. We hypothesized that the tear film concentrations and the duration of drug retention on the ocular surface will be greater when using a formulation with a high versus low concentration. Similarly, we hypothesized that tear film kinetics will be enhanced when repeating eye drop administration, and that the interval between the two separate eye drops would be an important factor. Ultimately, enhanced drug delivery could improve owner/patient compliance (e.g., lower frequency of administration) and clinical outcomes of canine patients.

2 | MATERIALS AND METHODS

2.1 | Animals

Six Beagle dogs were used for the study (3 neutered males and 3 spayed females), aged 2–3.5 years old and weighing 7.5–10 kg. All dogs were confirmed to be healthy based on complete physical and ophthalmic examinations by a board-certified veterinary ophthalmologist (LS, MK), including slit-lamp biomicroscopy (SL-17; Kowa Company, Ltd.), indirect ophthalmoscopy (Keeler Vantage; Keeler Instruments, Inc.), rebound tonometry (TonoVet; Icare Finland Oy), Schirmer tear test-1 (STT-1; Eye Care Product Manufacturing LLC), and fluorescein staining of the ocular surface (Flu-Glo, Akorn, Inc.). The study was approved by the Institutional Animal Care and Use Committee at Iowa State University (IACUC # 21-065).

2.2 | Experiments

Two ophthalmic solutions of fluorescein (0.3% and 1%) were prepared aseptically by a pharmacist by mixing 1.4% polyvinyl alcohol (Artificial Tear Solution®; Rugby) with 10% sodium fluorescein (AK-Fluor®, Akorn Inc.). Specific concentrations of 0.3% and 1% were chosen based on common commercially available ophthalmic medications (e.g., 0.3% ofloxacin, 0.3% tobramycin, 1% atropine, and 1% tropicamide). In each dog, one eye was randomly selected by coin toss to receive one drop (35 μL) of 0.3% fluorescein while the other eye received one drop of 1% fluorescein solution, and this choice was kept constant for all trials. Topical administrations and tear collections were performed by the same examiner (LP).

2.2.1 | 0.3% versus 1% solutions

Following topical instillation with a pipette (Eppendorf Reference® 2, 10–100 μL), tear fluid was collected from each eye with a 2-μL capillary glass tube (Drummond Scientific Co.) at the following time points: 0 min (i.e., immediately after instillation and spontaneous blinking), 1, 5, 10, 20, 30, 40, 50, 60, 90, 120, and 180 min.

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2.2.2 | Repeated administration

Each trial was performed on a separate day along with thorough eye rinsing at the end of each trial to avoid cross-over of fluorescein from one experiment to another. One eye drop (35 µL) was instilled in each eye, followed by another eye drop (35 µL) of the same solution after 30 s (Day 1), 1 min (Day 2), 2 min (Day 3), 5 min (Day 4), or 10 min (Day 5). Thereafter, tear fluid was collected from each eye with a 2-µL capillary glass tube at 0 min (i.e., immediately after instillation of the second eye drop and spontaneous blinking), 1, 5, 10, 20, 30, 40, 50, 60, 90, 120, and 180 min.

2.2.3 | Fluorescein quantification in tear samples

The length of fluid in each capillary tube was measured with a ruler to derive the volume of tears collected (2-µL tube equates to 32 mm in length). Then, tears were expelled from each tube into 2-mL Eppendorf tubes, with each containing 500 µL of phosphate-buffered saline (Gibco® PBS, pH 7.2, Thermo Fisher Scientific). Samples were vortexed for 30 s and transferred to a cuvette for analysis. Fluorescein concentrations were measured in ng/mL with a computerized scanning ocular fluorophotometer (Fluorotron Master™, Coherent Radiation) as previously described.[4,5]

2.3 | Data analysis

Precorneal retention time was defined as the time (in min) that fluorescence of the tear film sample decreased below 5% of the baseline value.[9] Following the linear-log trapezoidal rule, the R software (version 3.6.0) was used to calculate the area under the concentration-time curves from 0 to 180 min ($AUC_{0\text{-}180}$) for each fluorescein concentration (0.3% or 1%) in the control experiment (single eye drop) and each of the five trials, that is, repeated eye drop administration at 30-s, 1-, 2-, 5-, and 10-min interval. The normality of the data was assessed with the Shapiro–Wilk test. When comparing 0.3% and 1% fluorescein solutions for the control experiment, precorneal retention times were assessed using the Wilcoxon signed-rank test, while $AUC_{0\text{–}180}$ and tear film fluorescence at each time point (0–180 min) were assessed using paired *t*-tests. When comparing the control experiment with the five trials for each fluorescein concentration (0.3% or 1%), precorneal retention times were assessed using Kruskal–Wallis test, a one-way ANOVA was used to compare $AUC_{0\text{–}180}$ while a two-way repeated measures ANOVA with post hoc Holm-Sidak was used to compare tear film fluorescence at each time point (0–180 min). Statistical analyses were performed with SigmaPlot 14.5 (Systat software). *p* values < .05 were considered significant for differences in $AUC_{0\text{–}180}$ and precorneal retention times, while *p* values < .0083 (= 0.05/6) were considered significant for differences in tear film fluorescence at each time point (Bonferroni correction for repeated measures in 6 trials).

3 | RESULTS

3.1 | 0.3% versus 1% solutions

Tear film kinetics of 0.3% and 1% fluorescein solutions following the instillation of a single eye drop (i.e., no repeated administrations) are depicted in Figure 1. Tear film fluorescence was significantly higher at

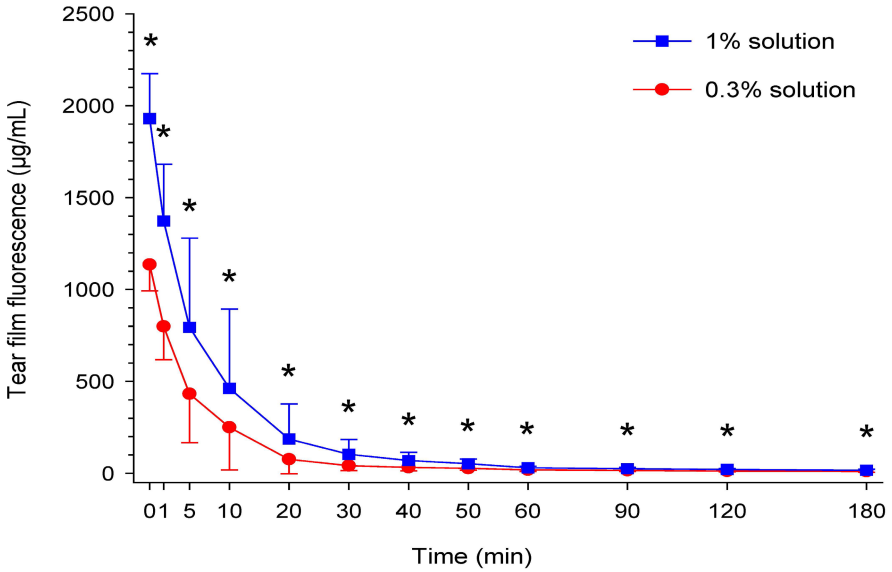

FIGURE 1 Mean ± SD fluorescein in tears from 0 to 180 min following topical administration of 0.3% or 1% fluorescein solution in eyes of 6 healthy Beagle dogs. At each time point, statistical differences between the groups are depicted by an asterisk (*).

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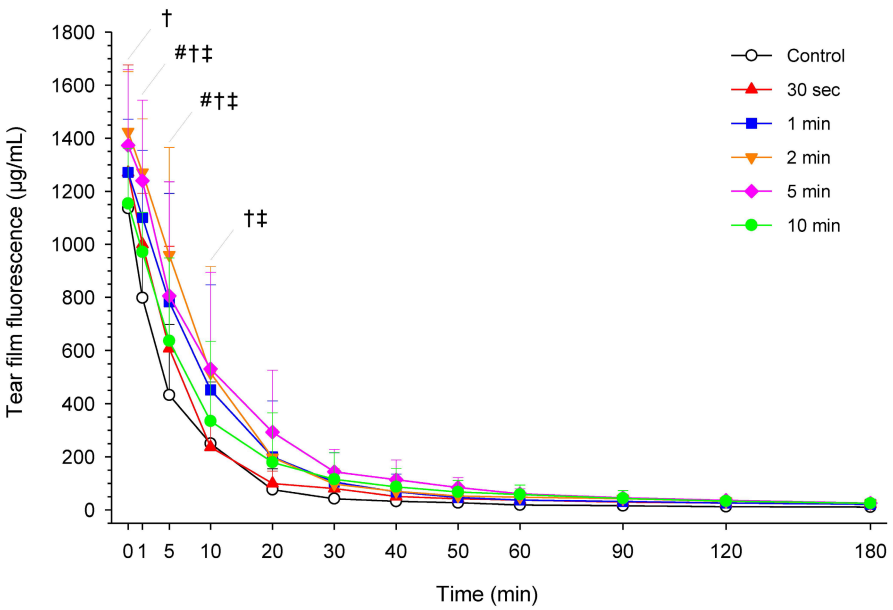

FIGURE 2 Mean + SD fluorescein in tears from 1 to 180 min following topical administration 0.3% fluorescein solution in eyes of 6 Beagles. Compared with control at each time point, statistically significant results are depicted by # for group 1 min; † for group 2 min; ‡ for group 5 min.

each time point (0–180 min) in eyes receiving 1% versus 0.3% fluorescein solution ($p \leq .046$). Furthermore, mean ± SD AUC_{0-180} was approximately twofold greater in eyes receiving 1% versus 0.3% fluorescein solution (18 367 ± 9018 vs. 9871 ± 4612 µg × min/mL), a finding that was statistically significant ($p = .005$). However, no significant difference was noted in median (range) precorneal retention time following a single drop of 0.3% or 1% fluorescein solution [15 (10–50) min vs. 25 (10–50) min, respectively; $p = 1.000$].

3.2 | 0.3% solution with repeated administration

Figure 2 depicts the tear film kinetics of 0.3% fluorescein solution following eye drop instillation at a single occasion (control) or two eye drop instillations separated by 30 s to 10 min (experimental trials). Statistical differences in tear fluorescence (0.3% solution) between experimental trials and control at each time point (0–180 min) are summarized in Table 1. Mean ± SD AUC_{0-180} (in µg × min/mL) in eyes receiving 0.3% fluorescein solution was superior in all groups [30-s (13 805 ± 4978), 1-min (17 986 ± 10 540), 2-min (20 668 ± 7891), 5-min (22 726 ± 9703), 10-min (17 549 ± 9084)] compared with control (9871 ± 4612); however, differences were not statistically significant ($p = .112$). Similarly, median and range (in min) precorneal retention time in eyes receiving 0.3% fluorescein solution was superior in all groups [30-s (40, 20–50), 1-min (35, 20–90), 2-min (40, 30–90), 5-min (55, 30–90), 10-min (45, 20–90)] compared with control (15, 10–50); however, differences were not statistically significant ($p = .160$).

TABLE 1 Statistical comparisons (two-way repeated measures ANOVA with post hoc Holm-Sidak) of tear film fluorescence at various times (0–180 min) in canine eyes receiving a single eye drop of 0.3% fluorescein solution (control) or two separate eye drops administered at 30 s, 1, 2, 5, and 10 min intervals.

Time (min)	Statistical results
0	2 min > control ($p = .002$)
1	1 min > control ($p < .001$)
	2 min > control ($p < .001$)
	5 min > control ($p < .001$)
5	1 min > control ($p < .001$)
	2 min > control ($p < .001$)
	5 min > control ($p < .001$)
10	2 min > control ($p = .004$)
	5 min > control ($p = .003$)
20	None ($p \geq .035$)
30	None ($p \geq .628$)
40	None ($p \geq .817$)
50	None ($p \geq .851$)
60	None ($p \geq .819$)
90	None ($p \geq .867$)
120	None ($p \geq .864$)
180	None ($p \geq .897$)

Note: Level of significance $\alpha = .0083$ (Bonferroni correction for repeated measures).

3.3 | 1% solution with repeated administration

Figure 3 depicts the tear film kinetics of 1% fluorescein solution following eye drop instillation at a single occasion

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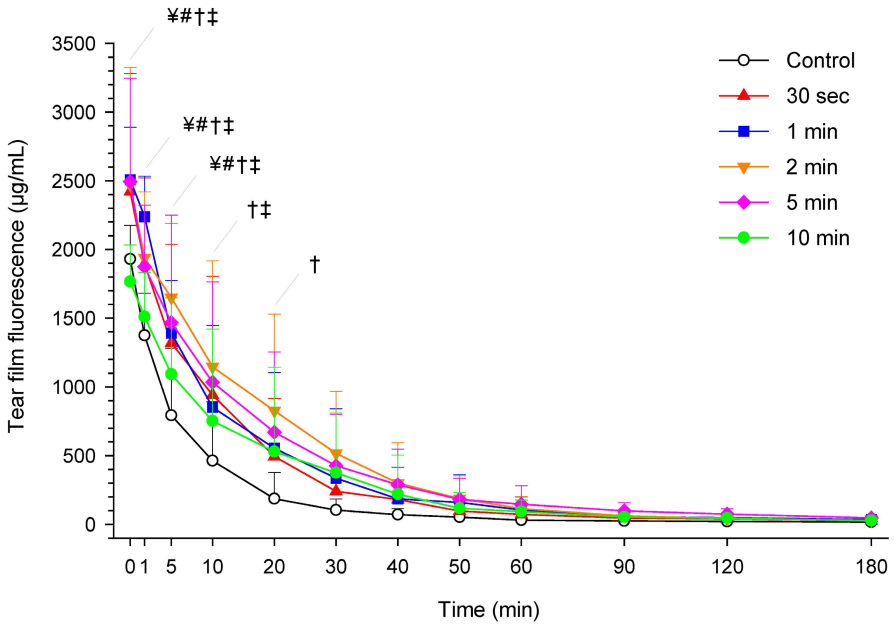

FIGURE 3 Mean + SD fluorescein in tears from 1 to 180 min following topical administration 1% fluorescein solution in the eyes of 6 Beagles. Compared with control at each time point, statistically significant results are depicted by: ¥ for group 30 s; # for group 1 min; † for group 2 min; ‡ for group 5 min.

TABLE 2 Statistical comparisons (two-way repeated measures ANOVA with post hoc Holm-Sidak) of tear film fluorescence at various times (0–180 min) in canine eyes receiving a single eye drop of 1% fluorescein solution (control) or two separate eye drops administered at 30 s, 1, 2, 5, and 10 min intervals.

Time (min)	Statistical results
0	30 s > control ($p = .006$)
	1 min > control ($p = .003$)
	2 min > control ($p = .003$)
	5 min > control ($p = .003$)
1	30 s > control ($p = .005$)
	1 min > control ($p < .001$)
	2 min > control ($p = .003$)
	5 min > control ($p = .005$)
5	30 s > control ($p = .003$)
	1 min > control ($p = .001$)
	2 min > control ($p < .001$)
	5 min > control ($p < .001$)
10	2 min > control ($p < .001$)
	5 min > control ($p = .003$)
20	2 min > control ($p < .001$)
30	None ($p \geq .062$)
40	None ($p \geq .500$)
50	None ($p \geq .786$)
60	None ($p \geq .800$)
90	None ($p \geq .901$)
120	None ($p \geq .906$)
180	None ($p \geq .954$)

Note: Level of significance $\alpha = .0083$ (Bonferroni correction for repeated measures).

(control) or two eye drop instillations separated by 30 s to 10 min (experimental trials). Statistical differences in tear fluorescence (1% solution) between experimental trials and control at each time point (0–180 min) are summarized in Table 2. Mean ± SD AUC_{0-180} (in µg × min/mL) in eyes receiving 1% solution was superior in all groups [30-s (34 466 ± 19 795), 1-min (38 769 ± 23 604), 2-min (47 290 ± 26 650), 5-min (46 537 ± 28 332), 10-min (33 963 ± 22 487)] compared with control (18 367 ± 9018); however, differences were not statistically significant ($p = .280$). Similarly, median and range (in min) precorneal retention time in eyes receiving 1% solution was superior in all groups [30-s (45, 20–90), 1-min (40, 20–90), 2-min (50, 40–120), 5-min (50, 10–180), and 10-min (35, 20–90)] compared with control (25, 10–50); however, differences were not statistically significant ($p = .255$).

4 | DISCUSSION

In dogs, tear film concentrations can be enhanced by increasing drug concentration in the ophthalmic formulation, and/or repeating topical administration shortly after the first eye drop. These strategies could be used by veterinarians and owners to improve the efficacy of topical drug administration to canine eyes. More specifically, the optimal time interval between two separate eye drops appeared to be 2–5 min in dogs, extending precorneal retention time by up to 3.7-fold and twofold (0.3% and 1% solutions, respectively), and improving the overall drug exposure by up to 130% and 157% (0.3% and 1% solutions, respectively). In contrast, there was no apparent benefit in repeating eye drop administration 30 s or 1 min after the

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first eye drop – possibly owing to excessive nasolacrimal drainage or spillage over the periocular skin (e.g., volume exceeding the capacity of the ocular surface) – nor 10 min after the first eye drop, likely because there is very little of the first eye drop left in the tear film by that time.[5,6]

Although the present findings should be verified in future studies that would assess different drug classes, it is reasonable to speculate on the potential benefits of the aforementioned strategies in clinical practice. As shown for mucoadhesive polymers and other tools,[10,11] improved ocular drug delivery might hasten and prolong the biological effects of common ophthalmic medications such as mydriatics for examination purposes or pre-surgical preparation (e.g., prior to phacoemulsification), miotics for lens instability or post-transcorneal lens reduction, hypotensive drugs for glaucoma management, anti-inflammatory or immunomodulatory drugs for keratoconjunctivitis or uveitis, as well as antimicrobials for patients with infectious keratitis. The latter is of particular importance given the intensive therapy most cases of bacterial keratitis require (e.g., two different antibiotics, up to every 1–2 h daily),[12] and the resulting burden on owners and patients' compliance. By increasing tear film concentrations, both strategies would likely improve pharmacokinetic-pharmacodynamic indices that dictate antibacterial efficacy, including time above minimal inhibitory concentrations ($t > \text{MIC}$), ratio of area-under-the-curve to MIC (AUC/MIC), and ratio of peak concentration to MIC (peak/MIC).[13] For instance, the twofold increase in AUC_{0-180} obtained with 1% versus 0.3% ophthalmic solution would likely enhance the potency of antibiotics for which AUC/MIC ratio is a major determinant of the activity (e.g., fluoroquinolones, aminoglycosides).[13] Similarly, the longer precorneal retention time obtained with higher concentration or with repeated eye drop administration would likely enhance the potency of antibiotics for which $t > \text{MIC}$ is an important determinant of the activity (e.g., β-lactams, macrolides).[13] In practice, the following steps could be taken to improve topical antibiotic delivery in dogs with bacterial keratitis. First, whenever available, preference should be given to ophthalmic formulations with higher antibiotic concentration; for instance, at the corresponding author's country of residence, topical chloramphenicol is commercially available as either a 0.2% solution (Phenimixin®, Vitamed Ltd.) or 0.8% solution (Crotax®, Caranixe, Portugal). Second, clinicians could consider fortified antibiotics by mixing commercially available ophthalmic solution with an injectable formulation of the same antibiotic[12]; for instance, 1.4% gentamicin solution can be obtained by adding 2 mL of 40 mg/mL injectable gentamicin to a 5-mL bottle of 0.3% gentamicin ophthalmic solution.[12] On the same note, an ophthalmic preparation of cefazolin solution can be readily prepared by mixing artificial tears with cefazolin powder, resulting in a high concentration of cefazolin (up to 5.5% depending on the recipe) that is pharmacologically desirable without apparent toxicity or interference with corneal epithelial wound healing.[14] Third, repeated eye drop administration could be performed at the start of antimicrobial therapy, similar to the "loading dose" regimen recommended by the American Academy of Ophthalmology,[15] and possibly at each dosing session to further enhance antibacterial coverage, especially if clients are unable to administer medication as often as ideally recommended.

The strategies described in the present study might also improve ocular drug delivery by minimizing the negative impact of tear film albumin on ocular bioavailability. In clinical patients, a breakdown of the blood-tear barrier allows for excess serum albumin to leak from the blood compartment to the tear film.[16–19] The resulting albumin-drug binding in tears reduces antimicrobial efficacy as only the unbound fraction of an antibiotic is microbiologically active,[20] and also lowers intra-ocular bioavailability as only unbound drugs can penetrate through the cornea.[21] A more concentrated ophthalmic formulation could possibly compensate for the fraction of drug "lost" to albumin binding in tears, whereas with repeated administration, the first eye drop could reduce the levels of unbound albumin in tear fluid and thereby improve the bioavailability of the second eye drop applied shortly after.

Importantly, ophthalmic solutions made up in a hospital or practice setting should be considered as compounded formulations and, therefore, follow relevant guidelines and regulations.[22] Further, although some antibiotic preparations may retain potency for relatively long periods,[23–25] compounded formulations should generally be considered "unstable" and be stored refrigerated for no more than a couple of days to weeks.[12,26,27] Lastly, higher drug concentration on the ocular surface could be irritating or cytotoxic to the corneal epithelium, and thereby negatively impact corneal wound healing. This was true for 2% gentamicin but not for other antibiotics tested in one study (e.g., 10% piperacillin, 5% cefazolin, 5% vancomycin, 1% amikacin, 0.5% chloramphenicol),[14] although further studies are needed for different antibiotic classes at various concentrations.

The present work was limited to fluorescein quantification in the tear film, and not ocular tissues, intraocular fluids (aqueous, vitreous), or plasma (systemic absorption). Therefore, we cannot comment on the potential systemic toxicity of the proposed strategies, and we can only speculate that the pharmacological benefits observed in the tear film would translate to clinical benefits in patients with corneal or intraocular pathologies. In humans, a topical fluoroquinolone at 1.5% concentration achieved higher drug levels in the cornea[7] and aqueous humor[7,8] compared with other fluoroquinolones at

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0.3–0.5% concentrations. In rabbits, a loading dose of topically applied clarithromycin (i.e., one drop instilled 6 times at 5-min intervals) provided significantly higher drug levels in the cornea and aqueous humor compared with single antibiotic dosing.[28] Another study limitation is the focus on fluorescein and only two different concentrations. As such, findings cannot be directly extrapolated to ophthalmic formulations that have different physicochemical properties (e.g., viscosity, lipophilicity, molecular weight) nor to ophthalmic formulations with drug levels other than 0.3% and 1%. Lastly, the study did not objectively evaluate tear film fluorescence at 24 h, that is, before starting a new experimental day; as such, we cannot rule out potential "carry over" of fluorescence from one experiment to another, although we believe it would have been extremely minimal (if any) and not clinically relevant. Even without accounting for the thorough ocular rinsing, fluorescein tear concentrations that were already very low at the last sampling time (<0.01–0.05 mg/mL at $t = 3$ h) would be expected to be even lower at $t = 24$ h owing to continued clearance over time, and thereby represent an insignificant fraction of fluorescein tear concentrations at $t = 0$ min (1.1–2.5 mg/mL).

In conclusion, the present study demonstrates that drug delivery to the canine ocular surface can be enhanced by using more concentrated formulations and/or by repeating eye drop administration 2–5 min after the first dose, improving overall drug exposure by up to 157%.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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