

# Tear film concentrations of doxycycline following oral administration in ophthalmologically normal dogs

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## OBJECTIVE

To determine tear film concentrations of doxycycline in ophthalmologically normal dogs following oral doxycycline administration.

## DESIGN

Crossover study.

## ANIMALS

10 privately owned dolichocephalic or mesocephalic dogs free of ophthalmic disease.

## PROCEDURES

Dogs were randomly assigned to receive doxycycline hyclate first at 5 mg/kg (2.3 mg/lb) or 10 mg/kg (4.5 mg/lb), PO, every 12 hours for 5 days, beginning on day 1. Doxycycline was administered 1 hour prior to feeding. Tear samples were collected from days 1 through 10 approximately 3 hours after the morning dose was administered. Following a 3-week washout period, dogs received the alternative dose in the same conditions. Doxycycline concentration in tear samples from 1 eye (same eye used for both sessions) was measured via liquid chromatography–mass spectrometry and compared between the 2 doxycycline doses.

## RESULTS

Doxycycline was detected in tear samples of all dogs from days 1 through 10 for both doxycycline doses. Median peak doxycycline concentrations for the 5 mg/kg and 10 mg/kg doses were 2.19 ng/mL on day 3 and 4.32 ng/mL on day 4, respectively. Concentrations differed significantly with time, but this difference was not influenced by dose, dose order, or eye. A significant positive correlation was identified between doxycycline concentration and body weight ( $r = 0.22$ ).

## CONCLUSIONS AND CLINICAL RELEVANCE

Detectable doxycycline concentrations were achieved in the tear film of ophthalmologically normal dogs following oral administration of doxycycline at 5 or 10 mg/kg, every 12 hours. Dose had no significant effect on tear film concentration of the drug. (*J Am Vet Med Assoc* 2016;249:508–514)

**D**oxycycline hyclate is a semisynthetic derivative of oxytetracycline, with superior lipophilicity and tissue penetration to that of tetracycline hydrochloride. Doxycycline hyclate is effective against both gram-positive and gram-negative bacterial infections by reversibly binding the 30S ribosomal subunit of infecting organisms, thus inhibiting bacterial protein synthesis.<sup>1</sup> Beyond antimicrobial activity, tetracyclines have anti-inflammatory and immunomodulatory properties with therapeutic benefit for the

treatment of ocular diseases such as corneal malacia, spontaneous chronic corneal epithelial defects (also known as indolent or nonhealing corneal ulcers), nodular granulomatous episcleritis, and nodular granular conjunctivitis.<sup>2–5</sup>

Spontaneous chronic corneal epithelial defects in dogs have been well described.<sup>6–9</sup> Clinical findings include superficial corneal ulceration with nonadherent edges of epithelium. The lack of proper epithelial adhesion to the underlying stroma is believed to be caused by a loss of hemidesmosomes. Numerous treatment methods, both surgical and nonsurgical, have been reported with varying success rates.<sup>10–14</sup> Recently, it has been suggested that treatment with topically or orally administered tetracyclines, in com-

## ABBREVIATIONS

CV Coefficient of variation  
LC-MS Liquid chromatography–mass spectrometry

ination with a grid keratotomy, may decrease the time required for corneal wounds to heal. The proposed mechanism underlying this effect is upregulation of transforming growth factor  $\beta$ , promoting migration of corneal epithelial cells.<sup>4</sup>

Keratomalacia, or corneal melting, can be a sterile or infectious process mediated by gelatinases, collagenases, and proteinases.<sup>15,16</sup> Depending on the severity, this process can result in considerable loss of corneal stroma, corneal perforation with loss of vision, or loss of the globe. Collagenases and proteinases are secreted by neutrophils within the precorneal tear film, microorganisms, and corneal epithelial cells.<sup>15</sup> Treatments for keratomalacia are aimed at enzymatic inhibition via administration of solutions to the ocular surface or parenteral administration of drugs that may enter the precorneal tear film. These treatments include autologous serum, EDTA, *N*-acetylcysteine, and tetracyclines for oral or topical administration.<sup>17-19</sup> Doxycycline has been administered orally for the adjunctive treatment of keratomalacia in several species, including horses, rabbits, and humans.<sup>20-22</sup>

Matrix metalloproteinases are categorized into several subgroups, including collagenase, gelatinase, and stromelysin. These enzymes are responsible for many physiologic and pathophysiologic processes within the body. Matrix metalloproteinases 2 and 9 are of the gelatinase group and play a key role in degradation of collagen type IV.<sup>23</sup> Activities of these 2 enzymes reportedly increase with wounding of the cornea in several species.<sup>5,24,25</sup>

Doxycycline has the ability to decrease the activity of matrix metalloproteinases *in vivo* in dogs and *in vitro* in horses.<sup>17,26</sup> Tetracyclines are believed to inhibit matrix metalloproteinase activity by chelation of structural and catalytic zinc within the enzyme. Doxycycline has the greatest ability to inhibit matrix metalloproteinases because of its higher affinity for zinc, compared with that of tetracycline hydrochloride and minocycline.<sup>23</sup>

When administered systemically, doxycycline can reach the tear film in several species, including horses,<sup>21</sup> cats,<sup>27</sup> and northern elephant seals.<sup>28</sup> The purpose of the study reported here was to determine whether doxycycline could be detected in the corneal tear film of ophthalmologically normal dogs following oral administration. We hypothesized that doxycycline would be detected in the tear film in these circumstances and that administration of doxycycline at 10 mg/kg (4.5 mg/lb), PO, every 12 hours would result in a significantly greater concentration of doxycycline in tear samples, compared with administration of doxycycline at 5 mg/kg (2.3 mg/lb), PO, every 12 hours.

## Materials and Methods

### Dogs

Dogs brought to the University of Illinois veterinary teaching hospital from September 2013 through October 2013 were eligible for the study if they were

free of ophthalmic and systemic disease, were of the dolicocephalic or mesocephalic skull type, weighed between 15 and 40 kg (33 and 88 lb), and were between the ages of 2 and 10 years. Dogs were enrolled until the target sample size ( $n = 10$ ) was achieved. This sample size had been determined by use of an  $\alpha$  value of 0.05, power of 0.80, expected mean difference between the 5 mg/kg and 10 mg/kg groups of 1.0 ng of doxycycline/mL of tear sample, and SD for each group of 0.75 ng of doxycycline/mL.

Informed consent was obtained from all dog owners prior to enrollment. All dogs received a complete ophthalmic examination by a board-certified veterinary ophthalmologist (ALL and REH), including a Schirmer tear test,<sup>a</sup> fluorescein staining,<sup>b</sup> rebound tonometry,<sup>c</sup> and slit-lamp biomicroscopy.<sup>d</sup> Pharmacologic mydriasis was provided with 1% tropicamide solution<sup>e</sup> to allow performance of indirect ophthalmoscopic<sup>f</sup> examination. A complete physical examination, CBC, serum biochemical analysis, and urinalysis were performed to ensure all dogs were free of systemic disease. All procedures were performed in compliance with the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research. The study protocol was approved by the Institutional Animal Care and Use Committee of the University of Illinois.

### Experimental protocol

Dogs were randomly allocated by use of a random number generator to 2 groups of 5 dogs each. One eye was chosen at random for tear film collection and used for the duration of the study. On day 0, a tear sample was collected from the selected eye by use of a Schirmer ophthalmic strip<sup>g</sup> placed in the ventral conjunctival fornix, allowing 15 mm of wetting to be obtained. Each wetted ophthalmic strip was placed in a 1.5-mL Eppendorf<sup>h</sup> tube, which had been weighed prior to strip collection and was weighed again once the strip had been added.

In phase 1 of the study, one dog group received doxycycline hyclate<sup>i</sup> at 5 mg/kg, PO, every 12 hours for 5 days and the other group received a 10 mg/kg dose, PO, every 12 hours for 5 days, beginning on day 1. Doxycycline was administered 1 hour prior to feeding each time. A tear sample was collected daily from days 1 through 10 approximately 3 hours after the morning doxycycline dose had been administered. A washout period of 3 weeks was provided after this phase ended, then phase 2 began, in which dogs received the alternative dose of doxycycline in the same administration and sample collection conditions as in phase 1. All tear samples were frozen at  $-80^{\circ}\text{C}$  until analysis.

### Measurement of doxycycline concentration in tear samples

Schirmer tear test strips were cut into small pieces within the Eppendorf tube, and 150  $\mu\text{L}$  of 60% methanol and 1  $\mu\text{L}$  of the internal standard demeclo-

cycline<sup>j</sup> (3.5 µg/mL) were added. Tubes were vortex mixed and centrifuged at 16,100 X *g* for 10 minutes. Supernatant was harvested for LC-MS analysis.

The high-performance LC-MS equipment<sup>k,l</sup> included a degasser, autosampler, and binary pump. Liquid chromatography separation was performed on a small-molecule separation column<sup>m</sup> with mobile phase A (0.1% formic acid in water) and mobile phase B (0.1% formic acid in acetonitrile). The flow rate was 0.3 mL/min. The linear gradient was as follows: 0 to 2 minutes, 100% A; 6 to 16 minutes, 0% A; and 16.5 to 22 minutes, 100% A. The autosampler was set at 5°C. The injection volume was 10 µL. Mass spectra were acquired with positive electrospray ionization, and the ion spray voltage was 5,500 V. The source temperature was 450°C. Parameter values for the curtain gas, ion source gas 1, and ion source gas 2 were 32, 50, and 65, respectively. Multiple reaction monitoring was used to quantify doxycycline (m/z 445.3 to 428.2), with demeclocycline (m/z 465.2 to 448.2) as the internal standard.

The quantitative LC-MS method was validated by measuring the consistency of results, correlation of results, and extraction efficiency of the assay. Within-run precision was calculated by use of 3 control samples (0.21, 2.1, and 17.5 ng of doxycycline/mL) repeated 6 times in a single run. Between-run precision was determined by comparing results for 3 control samples (0.21, 2.1, and 17.5 ng of doxycycline/mL) over 3 consecutive daily runs. The assay accuracy for within runs and between runs was estimated by determining the ratio of calculated response to expected response for previously measured control samples.

Long-term stability of doxycycline and demeclocycline concentrations in tear samples during storage was assessed by use of blank Schirmer tear test strips with spiked standards (demeclocycline at 2.33 ng/mL; doxycycline at 0.21, 2.1, and 17.5 ng/mL). The spiked samples were stored at -80°C for 192 days. These samples were processed on the day of testing and run together with samples extracted from blank Schirmer tear test strips spiked with freshly prepared standards.

## Statistical analysis

The distribution of the data was evaluated by use of the Shapiro-Wilk test, evaluation of skewness and

kurtosis, and creation of Q-Q plots.<sup>n</sup> Nonnormally distributed data were logarithmically transformed for parametric analysis. Mean ± SD and range are reported for normally distributed data, whereas median, 10th to 90th percentiles, and range are reported for nonnormally distributed data.

A linear mixed model to account for the crossover study design and a double dose that 1 dog received during phase 1 was used to evaluate the doxycycline concentrations over time. This allowed inclusion of the dog's day 1 result in the analysis. Dog was included in the model as a random factor, and dose order was included to evaluate the order of dose use. Dose, dose order, and eye were included as fixed factors evaluated in the model. The -2 log likelihood value was used to determine best fit of the model. The Pearson correlation test was used to determine whether doxycycline concentrations were correlated with dog body weight. Values of *P* < 0.05 were used to indicate significant differences.

## Results

### Dogs

Six female and 4 male dogs were enrolled and completed both phases of the study. Mean ± SD age was 5.3 ± 2.1 years (range, 2.9 to 9.1 years). Median body weight was 30.0 kg (66 lb; range, 15.3 to 38.5 kg [33.7 to 84.7 lb]). There were 6 mixed-breed dogs, 2 German Shorthaired Pointers, 1 German Shepherd Dog, and 1 Greyhound.

Tear samples were collected at a median time after morning dose administration of 181 minutes (range, 180 to 270 minutes) and 180 minutes (range, 160 to 211 minutes) for phases 1 and 2, respectively. A single episode of vomiting was identified for 3 of 10 dogs after receiving the 5 mg/kg dose of doxycycline and for 1 dog after it received a double dose (2 doses of 5 mg/kg each) once.

Within-run precision, between-run precision, and CVs of the LC-MS assay were determined (**Table 1**). Evaluation of long-term (192-day) stability of doxycycline concentrations in tear samples revealed that with spiked concentrations of 0.21, 2.1, and 17.5 ng/mL, percentages of analyte recovered were 93%, 78%, and 71%, respectively. For the evaluation of long-term sta-

**Table 1**—Mean ± SD values for accuracy and precision of an LC-MS assay for measurement of doxycycline concentrations in canine tear samples.

Doxycycline concentration (ng/mL)	Within-run accuracy (%)	Within-run CV (%)	Between-run accuracy (%)	Between-run CV (%)
0.21	106.1 ± 2.8	2.6	106.1 ± 2.8	2.6
2.1	99.9 ± 2.7	2.7	99.9 ± 2.7	2.7
17.5	104.2 ± 4.9	4.7	104.2 ± 5.0	4.8

Within-run precision (CV) was calculated from similar responses from 6 repeated analyses of 3 control samples (0.21, 2.1, and 17.5 ng/mL) in 1 run. Between-run precision (CV) was determined by comparing the calculated response of the low (0.21 ng/mL), medium (2.1 ng/mL), and high (17.5 ng/mL) concentration control samples over 3 consecutive daily runs (total of 6 runs). Assay accuracy for within and between runs was established by determining the ratio of the calculated response to the expected response for the 3 concentrations of control samples over 6 runs.

bility of demeclocycline concentrations, percentage of analyte recovered was 68%.

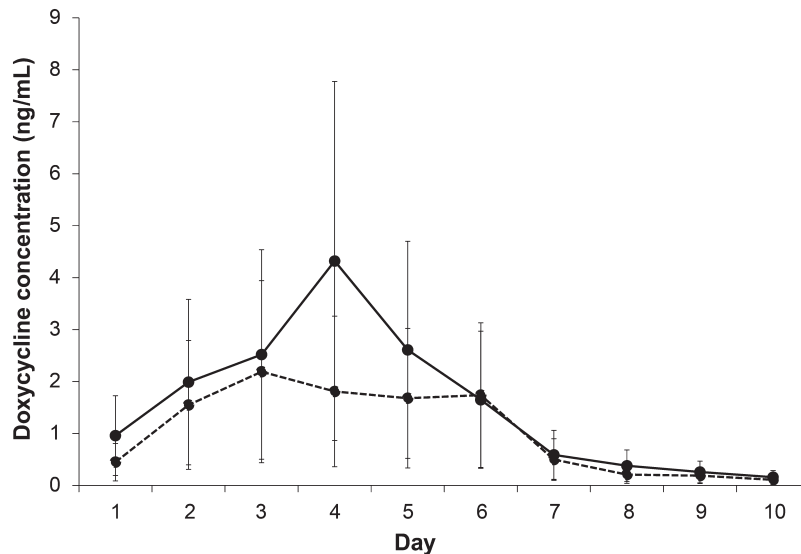
Doxycycline was detected in tear samples of all dogs between days 1 and 10 during both phases (**Figure 1**). Median peak concentrations of doxycycline in tear samples for the 5 mg/kg and 10 mg/kg doses were 2.19 ng/mL on day 3 and 4.32 ng/mL on day 4, respectively (**Table 2**). A significant ( $P < 0.001$ ) difference in doxycycline concentrations over time was identified, but the difference was not

influenced by dose ( $P = 0.13$ ), dose order ( $P = 0.52$ ), or eye ( $P = 0.70$ ). A significant ( $P = 0.002$ ) positive correlation was detected between doxycycline concentrations and dog body weight ( $r = 0.22$ ).

## Discussion

The results of the study reported here suggested that doxycycline hyclate administered orally to ophthalmologically normal dogs could be detected within the tear film. These dogs were intentionally chosen to reduce variability associated with tear film composition. Ocular diseases, including corneal ulceration, meibomian gland dysfunction, and qualitative or quantitative tear film deficiencies, alter tear film composition or rates of tear secretion, which may alter doxycycline concentrations within the tear film.<sup>5,23,29,30</sup> Brachycephalic dogs were also excluded from the study because, compared with other dogs, they have reduced corneal sensitivity,<sup>31</sup> prominent globes, and decreased tear film stability, which may increase the variability of tear film composition.<sup>32,33</sup>

The doses of doxycycline used in the present study were chosen on the basis of existing recommendations for doxycycline administration for the treatment of systemic disease.<sup>1</sup> No significant difference in tear doxycycline concentrations was identified between the 5 mg/kg and 10 mg/kg doses when administered PO every 12 hours, with peak concentrations achieved on days



**Figure 1**—Median doxycycline concentrations in tear samples from 10 ophthalmologically normal dogs at various points after receiving doxycycline hyclate at 5 mg/kg (2.3 mg/lb; dashed line) or 10 mg/kg (4.5 mg/lb; solid line), PO, every 12 hours for 5 days (beginning on day 1) in a crossover study design. Tear samples were obtained approximately 3 hours after the morning dose was administered. A washout period of 3 weeks was provided before beginning administration of the alternative dose. Error bars represent the 10th to 90th percentiles.

**Table 2**—Summary data for doxycycline concentrations (ng/mL) in tear samples from 10 dogs that received doxycycline hyclate at 5 mg/kg (2.3 mg/lb) or 10 mg/kg (4.5 mg/lb), PO, every 12 hours for 5 days (beginning on day 1) in a crossover study design.

Day	5 mg/kg			10 mg/kg		
	Median	10th–90th percentile	Range	Median	10th–90th percentile	Range
1	0.45	0.05–1.52	0.04–1.58	0.96	0.47–4.02	0.46–4.10
2	1.55	0.78–4.33	0.78–4.55	1.99	0.64–3.27	0.58–3.36
3	2.19	0.84–3.79	0.81–3.87	2.52	1.00–3.86	0.98–3.87
4	1.81	0.89–5.72	0.88–5.73	4.32	0.94–7.45	0.93–7.60
5	1.68	1.10–4.63	1.08–4.86	2.61	1.41–6.40	1.40–6.62
6	1.74	0.56–2.69	0.54–2.73	1.65	1.08–3.25	1.08–3.33
7	0.5	0.23–3.17	0.23–3.35	0.59	0.27–1.87	0.26–1.97
8	0.21	0.09–1.42	0.08–1.49	0.38	0.12–0.56	0.11–0.56
9	0.19	0.05–1.42	0.05–1.45	0.26	0.05–0.52	0.04–0.54
10	0.11	0.04–0.88	0.04–0.94	0.16	0.05–0.45	0.05–0.45

Tear samples were obtained approximately 3 hours after the morning dose was administered. A washout period of 3 weeks was provided before beginning administration of the alternative dose.

3 (2.19 ng/mL) and 4 (4.32 ng/mL), respectively. These concentrations were much lower than those reported for other species, including horses given doxycycline at 20 mg/kg, PO, once a day for 4 days (9.83 µg/mL)<sup>21</sup>; northern elephant seals given the drug at 20 mg/kg, PO (250 ng/mL at 4.1 hours after administration) or at 10 mg/kg, PO (170 ng/mL at 2.3 hours after administration)<sup>28</sup>; and cats given the drug at 5 mg/kg, PO (110 ng/mL at 4 hours after administration).<sup>27</sup>

Although not measured in the present study, serum doxycycline concentrations following oral administration of the drug have been reported for dogs, with a peak concentration of 2.53 µg/mL achieved 1.2 hours after administration.<sup>34</sup> Similar concentrations have been achieved in horses 1.5 hours after oral administration (1.74 µg/mL) and in northern elephant seals 2.3 hours after administration (2.4 µg/mL).<sup>28,35</sup> Given this information on serum concentrations in various species, there does not appear to be a direct correlation between serum and tear concentrations of doxycycline.

Long-term stability of doxycycline concentrations in stored tear samples was assessed in the present study to ensure that a clinically important amount of doxycycline hydrochloride would not be lost during sample storage prior to analysis. None of the evaluated concentrations decreased substantially during storage at -80°C for 192 days, with the greatest decrease identified following storage of samples containing the 17.5 ng/mL concentration (71% recovery). This decrease in doxycycline concentration with sample storage did not explain the lower concentrations of doxycycline in canine tear samples, compared with amounts reported for other species.

The ability of doxycycline to reach the tear film is hypothesized to be based primarily on the percentage of the drug that binds to plasma proteins, with reported plasma protein binding of 82% for horses,<sup>21</sup> 99% for cats,<sup>27</sup> and a value between these 2 percentages for northern elephant seals.<sup>28</sup> The reported percentage of plasma protein binding for doxycycline in dogs is 80% to 85%, and it has been suggested that protein binding plays the dominant role in relative distribution of tetracyclines.<sup>36,37</sup>

Given the similarities in values between dogs and horses, doxycycline concentrations in dogs may be expected to be similar to those in horses. Because tear film doxycycline concentrations in dogs of the present study were considerably lower than those measured in horses, other drug transport mechanisms or intrinsic factors may be involved that account for this difference. Doxycycline concentration, although diminished, could be detected in tear samples from all dogs in the present study for at least 5 days after administration was discontinued, which in other species is believed to be attributable to an accumulation of doxycycline within the lacrimal and meibomian glands.<sup>21,38,39</sup>

Activities of matrix metalloproteinases 2 and 9 in horses can be inhibited in vitro by numerous compounds, including 0.1% doxycycline.<sup>17</sup> In a study in-

volving 0.1% doxycycline,<sup>17</sup> tear samples were collected from horses with ulcerative keratitis and pooled together. Doxycycline solution was then added to tear samples, and matrix metalloproteinase activity was evaluated via gelatin zymography and measurement of optical density. Addition of 0.1% doxycycline to tear samples in that study<sup>17</sup> resulted in a 96.3% reduction in matrix metalloproteinase activity when the sample doxycycline concentration was 500 ng/mL. In another study,<sup>26</sup> however, 0.001% doxycycline administered topically for 2 days to ophthalmologically normal dogs resulted in only a 47% reduction in matrix metalloproteinase activity. The investigators in that study<sup>26</sup> suggested that the observed decrease in effectiveness could have been related to several factors, including the use of healthy subjects and possibly an inadequate concentration of doxycycline achieved in vivo, given that the dose they used had been extrapolated from an in vitro study.<sup>29</sup>

Tear doxycycline concentration in the present study reached a peak concentration of 4.32 ng/mL. Whether this low concentration would have the ability to decrease the activity of matrix metalloproteinase to a clinically meaningful degree for effective treatment of keratomalacia is unknown. This finding does, however, provide baseline information and a rationale for additional in vitro studies to evaluate different concentrations of doxycycline in canine tear samples.

*Staphylococcus* spp, *Streptococcus* spp, and *Pseudomonas* spp are the most common pathogenic organisms recovered from dogs with bacterial ulcerative keratitis.<sup>32,40</sup> Oral administration of doxycycline could be used to treat infections with these organisms primarily, or adjunctively, if a sufficiently high concentration could be attained within the tear film. We suspect that the low concentration of doxycycline identified in the tear film of dogs in the present study would not have been adequate to kill these organisms on the basis of the reported minimum inhibitory concentrations required to kill 90% percent of organisms.<sup>41-43</sup>

Administration of tetracyclines, in combination with a grid keratotomy, resulted in improved healing times for treating spontaneous chronic corneal epithelial defects of dogs in a previous study.<sup>4</sup> The mechanism proposed to underlie this improvement was a tetracycline-induced upregulation of the expression of transforming growth factor β, which would promote migration of corneal epithelial cells. Dogs that received a grid keratotomy in combination with topically administered oxytetracycline had a significantly shorter healing time than did control dogs. Dogs that received a grid keratotomy and orally administered doxycycline had improved healing times, but this improvement was not significantly different from the results for control dogs.<sup>4</sup>

For dogs in the present study, oral administration of doxycycline was performed every 12 hours for 5 days. Although no significant difference was identified between the 5 mg/kg and 10 mg/kg doses, the 10 mg/kg dose resulted in a slightly higher median concentra-

tion of doxycycline within the tear film. It remains unknown whether doxycycline administration at 10 mg/kg would have improved healing times for dogs within this study, but given the higher median concentration of doxycycline achieved with this higher dose, this possibility may merit additional investigation.

Three of 10 dogs in the present study had a single episode of vomiting, and for 2 of these dogs, it was with the higher dose (10 mg/kg). Each of these episodes happened during phase 1 of the study, while dogs were orally receiving doxycycline. Because vomiting occurred only once for affected dogs, they were not excluded from the remainder of the study. Evaluation of daily doxycycline concentrations in tear samples from these dogs revealed no noticeable decrease, so most, if not all, of the medication had likely already been absorbed prior to vomiting.

One of 10 dogs in the present study received an accidental double dose (5 mg/kg) of medication on 1 day but had no subsequent signs of gastrointestinal distress, and no difference in tear doxycycline concentration was identified for that day relative to the concentration in the remaining samples collected for that dog. Generally, doxycycline is safe and well tolerated by dogs, as was supported by the lack of major adverse effects in the study dogs.<sup>1</sup>

Findings of the study reported here suggested that doxycycline can reach the tear film of ophthalmologically normal dolicocephalic or mesaticephalic dogs following oral administration at 5 mg/kg or 10 mg/kg, every 12 hours for 5 days. Doxycycline administered in this manner may have the ability to inhibit matrix metalloproteinase activity in the tear film of dogs. Additional studies are needed to evaluate the ability of doxycycline to inhibit matrix metalloproteinase activity in the tear film of dogs with corneal ulceration at the tear concentrations achieved in the present study. Until those studies have been performed, we remain uncertain whether oral administration of doxycycline may be beneficial in the treatment of dogs with corneal diseases, including keratomalacia, infectious keratitis, nonhealing ulcers, and other keratopathies.

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## Footnotes

- a. Intervet Inc, Roseland, NJ.
- b. BioGlo fluorescein sodium ophthalmic strips USP, Ocularvision Inc, Solvang, Calif.
- c. Tonovet, Icare Finland OY, Espoo, Finland.
- d. Kowa-SL2, Kowa, Tokyo, Japan.
- e. Bausch and Lomb Inc, Tampa, Fla.
- f. Heine EN 20-1, Heine Optotechnik, Herrsching, Germany or Keeler Vantage, Keeler Instruments Inc, Broomall, Pa.

- g. Jorgensen Inc, Loveland, Colo.
- h. Eppendorf North America, Hauppauge, NY.
- i. Mutual Pharmaceutical Co Inc, Philadelphia, Pa.
- j. Sigma-Aldrich Corp, Atlanta, Ga.
- k. 5500 QTRAP LC-MS instrument, AB SCIEX, Foster City, Calif.
- l. 1200 series high performance liquid chromatography system, Agilent Technologies, Santa Clara, Calif.
- m. Zorbax SB-CN column (2.1 X 50 mm; 5 µm), Agilent Technologies, Santa Clara, Calif.
- n. SPSS, version 22.0, SPSS Inc, Chicago, Ill.

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## From this month's AJVR

### Effects of experimental cardiac volume loading on left atrial phasic function in healthy dogs

Tatsuyuki Osuga et al

#### OBJECTIVE

To elucidate the relationship between acute volume overload and left atrial phasic function in healthy dogs.

#### ANIMALS

6 healthy Beagles.

#### PROCEDURES

Dogs were anesthetized. A Swan-Ganz catheter was placed to measure mean pulmonary capillary wedge pressure (PCWP). Cardiac preload was increased by IV infusion with lactated Ringer solution at 150 mL/kg/h for 90 minutes. Transthoracic echocardiography was performed before (baseline) and at 15, 30, 45, 60, 75, and 90 minutes after volume loading began. At each echocardiographic assessment point, apical 4-chamber images were recorded and analyzed to derive time–left atrial area curves. Left atrial total (for reservoir function), passive (for conduit function), and active (for booster-pump function) fractional area changes were calculated from the curves.

#### RESULTS

Volume overload resulted in a significant increase from baseline in PCWP from 15 to 90 minutes after volume loading began. All fractional area changes at 15 to 90 minutes were significantly increased from baseline. In multiple regression analysis, quadratic regression models were better fitted to the relationships between PCWP and each of the total and active fractional area changes than were linear regression models. A linear regression model was better fitted to the relationship between PCWP and passive fractional area change.

#### CONCLUSIONS AND CLINICAL RELEVANCE

Results indicated that left atrial phasic function assessed on the basis of left atrial phasic areas was enhanced during experimental cardiac volume loading in healthy dogs. The effect of volume load should be considered when evaluating left atrial phasic function by indices derived from left atrial phasic sizes. (*Am J Vet Res* 2016;77:952–960)



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