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Population pharmacokinetics of doxycycline in the tears and plasma of northern elephant seals (*Mirounga angustirostris*) following oral drug administration

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AQUATIC ANIMALS

Objective—To assess tear and plasma concentrations of doxycycline following oral administration to northern elephant seals (*Mirounga angustirostris*).

Design—Pharmacokinetic study.

Animals—18 juvenile northern elephant seals without signs of ocular disease.

Procedures—Study seals were receiving no medications other than a multivitamin and were free from signs of ocular disease as assessed by an ophthalmic examination. Doxycycline (10 or 20 mg/kg [4.5 or 9.1 mg/lb]) was administered orally every 24 hours for 4 days. Tear and plasma samples were collected at fixed time points, and doxycycline concentration was assessed by means of liquid chromatography–tandem mass spectrometry. Concentration-time data were calculated via noncompartmental analysis.

Results—Following administration of doxycycline (10 mg/kg/d, PO), maximum plasma doxycycline concentration was 2.2 µg/mL at 6.1 hours on day 1 and was 1.5 µg/mL at 4.0 hours on day 4. Administration of doxycycline (20 mg/kg/d, PO) produced a maximum plasma doxycycline concentration of 2.4 µg/mL at 2.3 hours on day 1 and 1.9 µg/mL at 5.8 hours on day 4. Doxycycline elimination half-life on day 4 in animals receiving doxycycline at a dosage of 10 or 20 mg/kg/d was 6.7 or 5.6 hours, respectively. Mean plasma-to-tear doxycycline concentration ratios over all days were not significantly different between the low-dose (9.85) and high-dose (9.83) groups. For both groups, doxycycline was detectable in tears for at least 6 days following cessation of dosing.

Conclusions and Clinical Relevance—Oral administration of doxycycline at the doses tested in the present study resulted in concentrations in the plasma and tears of northern elephant seals likely to be clinically effective for treatment of selected cases of systemic infectious disease, bacterial ulcerative keratitis, and ocular surface inflammation. This route of administration should be considered for treatment of corneal disease in northern elephant seals and possibly other related pinniped species. (*J Am Vet Med Assoc* 2013;243:1170–1178)

Many captive marine mammal species, particularly pinnipeds, frequently develop severe, chronic, and recurrent ocular problems, especially corneal disease or

ABBREVIATIONS

AUC	Area under the concentration-time curve
Cl/F	Total body clearance as a function of bioavailability
Cmax	Maximum concentration
MIC	Minimum inhibitory concentration
NAD	Naïve-averaged data
NPD	Naïve-pooled data
STT	Schirmer tear test
Tmax	Time to reach maximum concentration

keratopathy.[1,a–d] Keratopathy in pinnipeds can manifest as corneal edema, bullous keratopathy, ulcerative or malacic keratitis, and even globe rupture which can result in pain and vision impairment. Similar clinical signs of disease in wild animals held temporarily in captivity during rehabilitation delay release of otherwise healthy animals or pose a major threat to their survival following release back into wild populations. Various causes of corneal disease in pinnipeds have been proposed, including infectious agents and environmental factors such as water quality, UV light, or toxins.[1,d] However, it is likely

that corneal disease in pinnipeds is multifactorial, and to date, no single underlying etiology has been proven.

Regardless of inciting cause, bacterial infection is a critical feature affecting the progression of ulcerative keratitis in pinnipeds. In terrestrial species, topical administration of an ophthalmic antimicrobial is the standard of care for corneal ulceration. However, in captive pinnipeds, topical ophthalmic drug administration can be challenging or impossible owing to the temperament of wild marine mammals, the aquatic environment that immediately dilutes the topical agent, and the requirement for frequent treatment. By contrast, oral administration of medications to pinnipeds is relatively simple and is safely and widely used in captive collections and rehabilitation settings. However, most orally administered drugs fail to reach the avascular cornea in terrestrial species. An orally administered antimicrobial that achieves adequate tear film concentrations in pinnipeds would provide a practical solution to managing an infected cornea and facilitate improved welfare and management of both captive and rehabilitating pinnipeds.

The purpose of the study reported here was to determine the population pharmacokinetics of doxycycline in tears and plasma following oral administration at 2 dosages to rehabilitating juvenile northern elephant seals (*Mirounga angustirostris*). Doxycycline was selected as the test drug because it is antimicrobial and anti-inflammatory and inhibits the matrix metalloproteinases responsible for corneal malacia and stromal loss.[2] Additionally, doxycycline becomes concentrated in the meibomian and lacrimal glands,[3] and even low-dose oral administration of doxycycline has been reported to be effective at treating meibomian gland dysfunction in humans.[4] Furthermore, doxycycline is frequently administered to pinnipeds and has a broad spectrum of activity against *Chlamydia* spp, *Mycoplasma* spp, *Rickettsia* spp, aerobic and anaerobic Gram-positive and -negative bacteria, *Brucella* spp, *Bartonella* spp, and *Leptospira* spp, among others.[5,6] Pinnipeds are commonly infected with many of those important and often zoonotic pathogens, particularly those in the genera *Leptospira, Brucella,* and *Bartonella*,[7–11] making doxycycline a justifiable antimicrobial choice in many instances. We hypothesized that doxycycline concentrations achieved in the tears and plasma of seals receiving 20 mg of doxycycline/kg (9.1 mg of doxycycline/lb) every 24 hours for 4 days would meet or exceed those necessary to have clinically relevant antimicrobial effects systemically and within the tear film as well as anti-inflammatory and antiprotease activity at the corneal surface. We further hypothesized that oral administration of 10 mg of doxycycline/kg (4.5 mg of doxycycline/lb) every 24 hours for 4 days to the same species would achieve therapeutic concentrations in plasma but not in tears.

Materials and Methods

Animals and study design—Eighteen juvenile northern elephant seals were included in this study. All animals were wild born and had been brought to The Marine Mammal Center, Sausalito, Calif, for rehabilitation because they were assessed in the field as malnourished. At the time of the study, the seals were nearing a typical weight for their age and were within a short time of being considered for release from The Marine Mammal Center back to their natural environment. This study was performed at The Marine Mammal Center and was approved by The Marine Mammal Center Animal Care and Use Committee and conformed to the guidelines of the Association for Research in Vision and Ophthalmology regarding animal use for ophthalmic research.[12] For inclusion in the present study, all seals were required to be receiving no medications other than a pinniped multivitamin[e] and free of signs of ocular disease as assessed by an ophthalmic examination, which included slit-lamp biomicroscopy and (if indicated) application of fluorescein dye.

Following baseline assessment, all seals were assigned to receive 1 of 2 dosages of doxycycline hyclate.[f] Seals in the low-dosage group (n = 6; 3 males and 3 females) received 10 mg of doxycycline/kg every 24 hours mixed with their ground fish mash and delivered by orogastric intubation. Seals in the high-dosage group (n = 12; 6 males and 6 females) received 20 mg of doxycycline/kg every 24 hours in a digestible gelatin capsule concealed within the coelomic cavity of a previously frozen herring. Seals in the high-dosage group were fed the remainder of their regular meal immediately after drug dosing. Seals in the low-dosage group were administered the drug via orogastric intubation because they were less robust than the other 12 seals and in some cases were not consistently eating on their own. The lower dosage was selected for the orogastric intubated seals to minimize any adverse effects from drug administration in these somewhat less robust wild animals intended for release. Animals capable of free feeding were selected for the high-dosage group. Numbers in each group were dependent on the number of seals fitting the inclusion criteria and available for assessment throughout the study period. Because the 2 different feeding methods may have affected drug absorption, data variability was minimized by maintaining a consistent feeding method within each dosage group, and neither feeding method nor dosage was changed throughout the study. All animals in both dosage groups received doxycycline with food at set times.

For both groups, doxycycline was administered at approximately 8:00 AM every day for 4 consecutive days. All 18 seals were housed in 3 groups of 6; animals within a group were fed in a uniform manner. Doxycycline dose was calculated on the basis of individual seal body weights obtained within 48 hours prior to administration of the first dose, and doxycycline powder was weighed to within 0.1 g of the calculated dose. Animals were reweighed within 96 hours following administration of the last dose of doxycycline.

Tear and blood sample collection—Tear and blood samples were collected according to a prescribed schedule following doxycycline administration. Seals required manual restraint for tear and blood sample collection. Therefore, wherever possible, tear and blood samples were collected concurrently, and the number of tear and blood samples and the timing of their collection from individual animals were designed so as to permit population pharmacokinetic assessment. For each group, blood samples were collected on days 1 and 4 at 1, 2, 4, 6, 8, and 24 hours after doxycycline administration. Tears were collected on days 1, 2, 3, 4, 5, 7, and 10 at 1, 2, and 4 hours after doxycycline administration. At each time point, tear and blood samples were collected from 3

animals from the low-dosage group and 4 animals from the high-dosage group. For example, 1 animal's path through the study would include an ophthalmic examination on day 0; doxycycline administration at 8:00 AM on days 1 to 4; blood and tear sample collection 1 hour later (9:00 AM) and blood sample collection 6 hours later (2:00 PM) on day 1; tear sample collection on days 2 to 5, 7, and 10 at 9:00 AM; and blood sample collection on day 4 at 9:00 AM and 2:00 PM. For the high-dosage group, 3 other animals had the same study schedule as this animal, and the remaining 8 animals were allocated into 2 groups and assigned to other sample collection schedules. Blood was collected into lithium heparin tubes from the extradural intervertebral sinus and centrifuged at 1,006 × g for 15 minutes, and the plasma was separated and stored at −20°C for 1 week and then −80°C until analyzed. Tears were collected by means of unmarked STT strips[g] as described[13] with minor modifications. Briefly, prior to tear collection, each STT strip was placed in a 2-mL cryovial and individually weighed. At the time of tear collection, the eyelids were gently dried with a 4 × 4-cm gauze pad (if needed), and the STT strips were removed from the cryovial with clean, dry, nontoothed forceps and placed into the ventral conjunctival fornix. Care was taken to ensure that the STT strip did not contact the eyelids so as to avoid absorption of any remaining pool water. The STT strip was left in place until at least half of the strip was wet, then placed into its original cryovial and stored at −20°C for 4 weeks. At that time, STT strips were reweighed in their cryovials, 1.0 mL of methanol was added to each cryovial, and all samples were stored at −80°C until analyzed.

Doxycycline quantification—Doxycycline concentration was assessed in all tear and plasma samples by liquid chromatography–tandem mass spectrometry. The liquid chromatography–tandem mass spectrometry conditions were selected on the basis of those described earlier,[14] with the liquid chromatography–tandem mass spectrometry system[h,i] in positive ion mode with electrospray ionization. All techniques were validated on a series of test samples. The methods used 40-μL aliquots of the tear extracts separated by a C18 guard column (3.0 × 7.5 mm; particle size, 3 μm) coupled to a C18 analytic column (3.0 × 50 mm; particle size, 3 μm) under gradient conditions. Doxycycline concentration was measured via liquid chromatography–tandem mass spectrometry for parent ion m/z 445 and product ion m/z 428 with simatone as the internal standard. The plasma and tear calibration samples were prepared in duplicate for each analytic run.

Calibration curves of doxycycline peak area ratios of the internal standard versus nominal concentration in plasma and tears were created. A weighting factor of 1/X was used to increase the accuracy. The calibration matrix curve was developed with the following predetermined FDA criteria: the mean value should be within ± 15% of the theoretical value, except at the lower limit of quantitation (± 20%) and the precision of mean value should not exceed a 15% coefficient of variation, except at the lower limit of quantitation (± 20%). The techniques were optimized to provide limits of detection of 0.1 and 0.05 ng/mL and limits of quantitation of 0.2 and 0.1 ng/mL for doxycycline concentrations in plasma and tears.

Pharmacokinetic analysis—Pharmacokinetic analysis of tear and plasma doxycycline concentrations was performed with commercial software.[j] Noncompartmental analysis of the tear and plasma data was performed with mean data for each time point. Linear trapezoidal areas were used to calculate the AUC, and other pharmacokinetic parameters were determined by use of standard noncompartmental equations. Specifically, the elimination rate constant was calculated as the slope of the terminal phase of the plasma-concentration curve that included a minimum of 3 points, and terminal elimination half-life was calculated as $0.693/k_{el}$, where k_{el} is the elimination rate constant. Compartmental analysis of the plasma data was performed via NPD and NAD approaches. The pharmacokinetic model selected was a 1-compartment pharmacokinetic model with first-order input and first-order output. Individual tear-to-plasma concentration ratios were calculated at all sampling points where both blood and tear samples were collected simultaneously. The AUC:MIC ratio for days 1 and 4 was calculated for the plasma samples at both doses for an MIC range of < 0.25 μg/mL.

Statistical analysis—Body weight was compared between groups and between study start and end with the Student t test for normally distributed data or the Mann-Whitney rank sum test when tests of normality were not met. Least squares linear regression was used to evaluate the relationship between tear and plasma doxycycline concentrations for the 10 and 20 mg/kg/d dosages. Significance was set at $P < 0.05$ for all analyses. Statistical analyses were performed with a commercial software package.[k]

Results

All northern elephant seals (n = 18) were approximately 6 months old. Median (range) body weight of seals in the low-dosage treatment group (37 kg [81.4 lb]; range, 32 to 42 kg [70.4 to 92.4 lb]) was significantly ($P < 0.01$) less than body weight of seals in the high-dosage treatment group (42.5 kg [93.5 lb]; range, 36 to 49.5 kg [79.2 to 108.9 lb]). Median body weight at baseline and following doxycycline administration did not differ significantly within the low-dosage group ($P = 0.70$) or the high-dosage group ($P = 0.07$). No seal in either treatment group demonstrated any adverse clinical signs attributable to drug administration at any time during the study.

Analysis of the plasma samples revealed that 1 animal in the low-dosage group had likely not received doxycycline on the first day of the study. Thus, this animal's plasma samples were not included in the pharmacokinetic analysis, reducing the total number of animals in the low-dosage group from 6 to 5. Tear doxycycline concentration data for the 4 days following the first dose in this seal (ie, days 2 to 5) were included (giving these days an n = 6) in the analysis but were adjusted to reflect time from administration of the first dose of doxycycline (day 2). Tear concentration data for the remaining days (days 1, 7, and 10) were excluded (therefore giving an n = 5 for these days).

Oral administration of 10 or 20 mg of doxycycline/kg resulted in measurable doxycycline concentrations in both plasma and tears of all 18 northern elephant seals. In

all animals, doxycycline was rapidly absorbed, with measurable concentrations in the plasma 1 hour after oral administration of a dose (**Figure 1**). Plasma pharmacokinetic variables for northern elephant seals receiving 10 or 20 mg
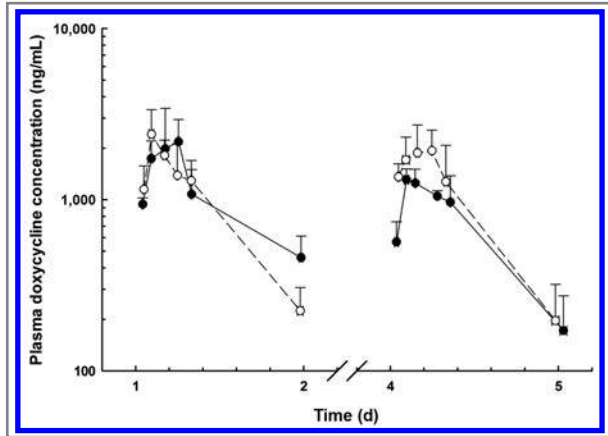

Figure 1—Mean + SD plasma doxycycline concentrations in northern elephant seals (*Mirounga angustirostris*) at various times following once-daily oral administration of 10 mg of doxycycline/kg (4.5 mg of doxycycline/lb) in ground fish mash delivered via orogastric intubation (black circles [n = 5]) or 20 mg of doxycycline/kg (9.1 mg of doxycycline/lb) in a digestible gelatin capsule concealed within the coelomic cavity of a previously frozen herring (white circles [12]). Daily administration of doxycycline started on day 1 and ended on day 4.

of doxycycline/kg PO once daily for 1 or 4 days were summarized (**Table 1**). Calculated pharmacokinetic variables and predicted plasma profiles for NAD and NPD modeling were similar for the high-dosage group on days 1 and 4 and for the low-dosage group on day 1 (**Table 2; Figure 2**). Calculated pharmacokinetic variables and predicted plasma profiles for NAD and NPD modeling varied slightly for the low-dosage group on day 4, with the data subjectively appearing to fit the NAD-predicted model more closely. The calculated plasma AUC:MIC ratio was > 100 for bacteria with an MIC ≤ 0.25 μg/mL on day 1 for both the high- and low-dosage groups and on day 4 for the high-dosage group only. The day 4 ratio for the low-dosage group was 76.

Doxycycline was detected in the tears of all 18 seals 1 hour after oral administration of a single dose of 10 or 20 mg of doxycycline/kg. On days 1 and 4, tear Cmax was greater in northern elephant seals receiving 20 mg of doxycycline/kg than in those receiving 10 mg of doxycycline/kg (Table 1). Tear doxycycline concentration in the high- and low-dosage treatment groups exceeded 100 ng/mL during the 4 days of oral dosing but decreased after drug administration was discontinued (**Figure 3**). A significant difference between the tear doxycycline concentrations of the 2 treatment groups was not detected on any day. Additionally, doxycycline was detectable in the tears 6 days after discontinuation of oral administration in 1 elephant seal from the

Table 1—Plasma and tear pharmacokinetic values determined by noncompartmental analysis on days 1 and 4 for northern elephant seals (*Mirounga angustirostris* [n = 17]) receiving 10 mg of doxycycline/kg (4.5 mg of doxycycline/lb; n = 5 for plasma, 6 for tears) in ground fish mash delivered via orogastric intubation or 20 mg of doxycycline/kg (9.1 mg of doxycycline/lb; 12) in a digestible gelatin capsule concealed within the coelomic cavity of a previously frozen herring PO once daily for 4 days.

	Day 1		Day 4	
Pharmacokinetic variable	**10 mg/kg**	**20 mg/kg**	**10 mg/kg**	**20 mg/kg**
Plasma				
Cmax (ng/mL)	2,200	2,400	1,500	1,900
Tmax (h)	6.1	2.3	4.0	5.8
Mean drug concentration in plasma (ng/mL)	—	—	770	1,000
Minimum drug concentration in plasma at steady state (ng/mL)	—	—	565	200
Fluctuation at steady state (%)	—	—	120	170
AUC (ng•h/mL)	31,000	26,000	19,000	26,000
Terminal elimination half-life (h)	9.1	6.5	6.7	5.6
Tears				
Cmax (ng/mL)	170	250	140	220
Tmax (h)	2.3	4.1	4.0	4.2
— = Not applicable.				

Table 2—Plasma pharmacokinetic values determined on days 1 and 4 by compartmental analysis and NPD or NAD modeling approaches for 17 northern elephant seals receiving 10 (n = 5) or 20 (12) mg of doxycycline/kg PO once daily for 4 days.

|	Day 1		Day 4	
Pharmacokinetic variable	**10 mg/kg**	**20 mg/kg**	**10 mg/kg**	**20 mg/kg**
NPD				
Absorption rate constant (h^{-1})	0.58	1.1	0.56	0.49
Elimination rate constant (h^{-1})	0.08	0.11	0.11	0.13
Apparent volume of distribution at steady state/bioavailability (L/kg)	4.0	7.1	4.9	6.0
Cl/F (mL/h/kg)	340	800	560	770
Biological half-life (h)	8.2	6.1	6.1	5.4
NAD				
Absorption rate constant (h^{-1})	0.82	0.76	0.46	0.51
Elimination rate constant (h^{-1})	0.08	0.12	0.094	0.13
Apparent volume of distribution at steady state/bioavailability (L/kg)	5.0	7.3	4.1	6.4
Cl/F (mL/h/kg)	380	840	390	830
Biological half-life (h)	9.1	6.0	7.4	5.4
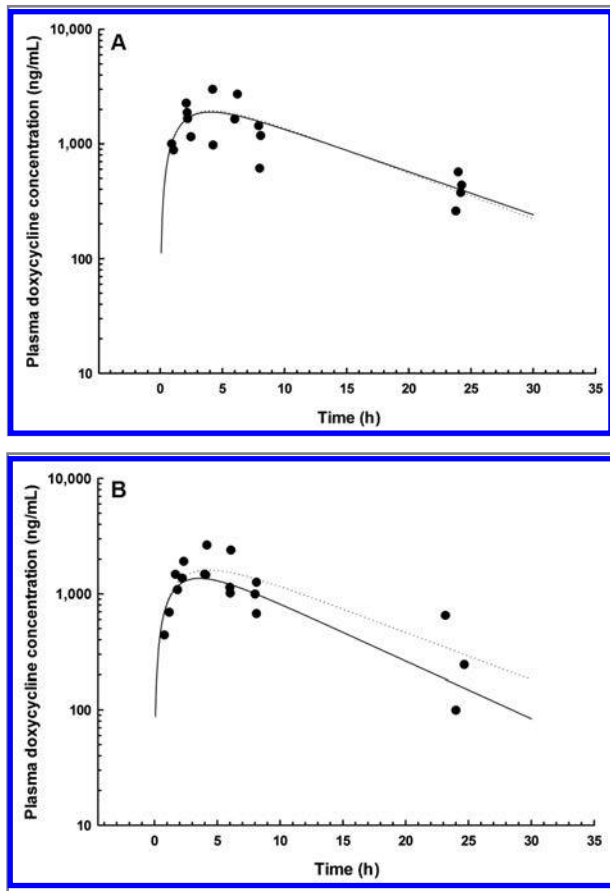
Figure 2—Plasma doxycycline concentrations as measured at various times (black circles) or predicted by means of NPD (solid lines) or NAD (dotted lines) modeling approaches in 5 northern elephant seals that received 10 mg of doxycycline/kg in ground fish mash delivered via orogastric intubation once daily for 4 days. A—Data following oral administration of a single dose of doxycycline (day 1). Note that predicted doxycycline concentrations are similar regardless of whether the NPD or NAD approach was used. B—Data following oral administration of the final dose of doxycycline (day 4). Note that doxycycline concentrations predicted using the NAD approach (dotted line) more predictably fit the data than do those predicted using the NPD approach (solid line).

low-dosage treatment group and in 10 of 12 seals in the high-dosage group. Following oral administration of a single dose of 10 or 20 mg of doxycycline/kg (ie, day 1 samples), mean ± SD tear doxycycline concentrations, expressed as a percentage of plasma doxycycline concentrations, were 9.9 ± 5.3% (range, 4.6% to 15.2%) or 11 ± 2.5% (range, 8.7% to 13.7%), respectively. Following administration of multiple doses of 10 or 20 mg of doxycycline/d (ie, day 4 samples), mean ± SD tear doxycycline concentrations were 9.8 ± 2.8% (range, 7.4% to 12.8%) and 8.7 ± 5.0% (range, 5.7% to 14.5%), respectively, of plasma doxycycline concentrations (**Figure 4**). A significant correlation between plasma and tear doxycycline concentrations was not detected in seals receiving 10 mg of doxycycline/kg ($P = 0.53$) or 20 mg of doxycycline/kg ($P = 0.29$; **Figure 5**).

Figure 4—Mean + SD plasma (circles) or tear (triangles) doxycycline concentrations in northern elephant seals at 1, 2, and 4 hours following once-daily oral administration of 10 mg of doxycycline/kg in ground fish mash delivered via orogastric intubation (black circles or triangles [n = 5 for plasma, 6 for tears]) or 20 mg of doxycycline/kg in a digestible gelatin capsule concealed within the coelomic cavity of a previously frozen herring (white circles or triangles [12]). Daily administration of doxycycline started on day 1 and ended on day 4.
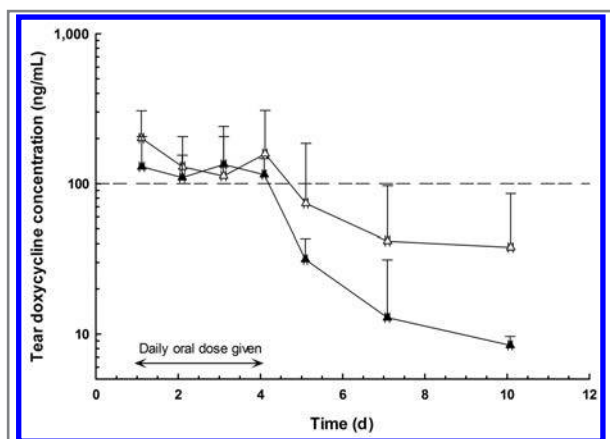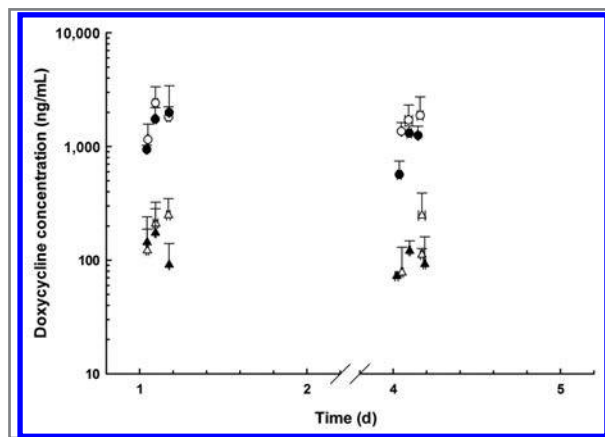

Figure 3—Mean + SD (combining hours 1, 2, and 4 of each day) tear doxycycline concentrations in northern elephant seals following once-daily oral administration of 10 mg of doxycycline/kg in ground fish mash delivered via orogastric intubation (black triangles [n = 6 for days 1 through 4 and 5 for days 5, 7, and 10]) or 20 mg of doxycycline/kg in a digestible gelatin capsule concealed within the coelomic cavity of a previously frozen herring (white triangles [12]) for 4 days. The horizontal dashed line at 100 ng/mL is the median MIC for some common doxycycline-susceptible bacteria (*Chlamydia* sp, *Mycoplasma* sp, *Bacillus* sp, *Streptococcus* sp, *Staphylococcus* sp).

Figure 5—Relationship between plasma and tear doxycycline concentrations in northern elephant seals following once-daily oral administration of 10 mg of doxycycline/kg in ground fish mash delivered via orogastric intubation (black squares [n = 5 for plasma, 6 for tears]) or 20 mg of doxycycline/kg in a digestible gelatin capsule concealed within the coelomic cavity of a previously frozen herring (white squares [12]) for 4 days. Data from days 1 and 4 were included. A significant correlation was not detected for the high-dosage ($P = 0.29$) or low-dosage ($P = 0.53$) group.

Discussion

Results of the present study, which involved a wild population of 18 northern elephant seals being rehabilitated at The Marine Mammal Center, indicated that once-daily oral administration of 10 or 20 mg of doxycycline/kg resulted in plasma and tear concentrations likely to be clinically effective for the treatment of selected cases of bacterial ulcerative keratitis and related ocular conditions associated with common pathogens (eg, *Leptospirosis, Brucella, Chlamydia, Streptococcus, Staphylococcus,* and some *Mycoplasma* spp) responsible for systemic and ocular disease in elephant seals. In addition, tear doxycycline concentrations were likely sufficient to have some antiprotease activity at the corneal surface. Although only limited observations of wellness were made and the treatment course was only 4 days, no adverse effects were observed in any seal receiving either dosage.

Although bioavailability of doxycycline in northern elephant seals was not directly calculated in this study, detectable plasma doxycycline concentrations within 1 hour after drug administration and observed plasma Cmax of 2,200 ng/mL (2.20 μg/mL) at 6.1 hours following oral administration of a single dose of 10 mg of doxycycline/kg were suggestive of adequate and relatively rapid drug absorption from the gastrointestinal tract. This Cmax is similar to that reported for sheep (2.13 ± 0.95 μg/mL),[5] although the Tmax was longer in these northern elephant seals (6.1 hours) than in sheep (3.6 ± 3.3 hours).[5] In the present study, doxycycline was administered with food to best replicate the clinical situation in this species, for which temperament precludes manual pill administration. However, in other species, feeding concurrent with doxycycline administration has been shown to alter drug bioavailability. For example, oral administration of doxycycline to chickens[15] and horses[16] from which food had been withheld resulted in a greater Cmax, shorter Tmax, and greater bioavailability than in fed animals, owing to increased absorption. Additionally, there were 2 administration routes (orogastric tube feeding vs gel-coated pill hidden in the coelomic cavity of fish) used in the present study, which could potentially have affected absorption. The seals in the low-dosage treatment group were being fed by orogastric intubation because they were less robust than the other 12 seals and in some cases were not consistently eating on their own. Nonetheless, all animals in both dosage groups received the doxycycline with food at uniform times. Additionally, the use of 2 feeding methods permitted us to generate data regarding 2 commonly used treatment modalities.

Following absorption, drugs with high lipophilicity such as doxycycline are usually distributed widely within tissues, especially fat, resulting in a large volume of distribution.[17] Indeed, the apparent volume of distribution (as a function of bioavailability) determined in northern elephant seals in the present study (4.0 to 7.1 L/kg [1.8 to 3.2 L/lb]) was large, especially in comparison to values for similarly sized mammals such as adult sheep (1.8 L/kg [0.8 L/lb]).[5] This large volume of distribution most likely occurs because doxycycline distributes into the elephant seals' subcutaneous fat (blubber), which is much thicker than that of terrestrial species. However, this comparison must be interpreted with caution because the oral bioavailability of doxycycline in northern elephant seals is not known. Intravenous administration is necessary to calculate the true volume of distribution, bioavailability, and clearance for any drug. This was not performed in the present study to minimize distress to the elephant seals and because IV doxycycline administration would be unlikely to be commonly used for this species in field conditions.

In other species, doxycycline is typically excreted via the kidneys. The elimination route of doxycycline is unknown in elephant seals and was not determined in the present study. However, the Cl/F of doxycycline in these northern elephant seals was 5.6 to 13.3 mL/kg/min (2.5 to 6.0 mL/lb/min), which is faster than the reported clearance in sheep (2.6 to 3 mL/kg/min [1.2 to 1.4 mL/lb/min]),[5] a similarly sized mammal. By comparison, the elimination half-life calculated in northern elephant seals following oral administration of a single dose of 20 mg of doxycycline/kg (6.5 hours) was similar to that in sheep (7.0 hours) receiving a single IV dose of 20 mg of doxycycline/kg.[5] The similar elimination half-life in these 2 species despite apparently faster clearance in northern elephant seals is likely explained by the larger volume of distribution observed in northern elephant seals versus sheep because elimination half-life is dependent on both distribution and elimination.

The present study also assessed the disposition of doxycycline in tears following oral drug administration to northern elephant seals. Appearance of doxycycline in the tears of these northern elephant seals was rapid, with measurable concentrations 1 hour after oral drug administration. Throughout the study period, tear doxycycline concentrations were approximately 10% of plasma concentrations. The concentration of antimicrobial compounds in tears is influenced by a number of intrinsic properties of the drugs (protein binding, lipophilicity, molecular weight, and degree of ionization), characteristics of the tears themselves (pH and flow rate), and transport mechanisms, such as diffusion and active transport.[18] Of these, plasma protein binding exerts a particularly important influence on a compound's distribution in interstitial fluids with more highly protein-bound compounds distributing less well into low-protein fluids such as tears than do less highly protein-bound compounds. The degree to which doxycycline is protein bound in northern elephant seals was not measured in the present study; however, data from other studies permit some hypotheses to be made. In cats, doxycycline is 99% protein bound and was not detectable in tears following oral administration of a single dose of 5 mg/kg (2.3 mg/lb).[18,19] By contrast, plasma protein binding of doxycycline after oral administration has been reported to be less in horses (82%) and was associated with a relatively high Cmax in tears (9,830 ng/mL) following administration of 20 mg of doxycycline/kg once daily.[3] The Cmax for doxycycline in northern elephant seal tears in the present study (250 ng/mL) was less than has been reported in horses (9,830 ng/mL)[3] but higher than in cats.[18] These prior studies of horses and cats served as the basis for the doses selected for the present study. As-

suming other factors are equal, findings from the present study in conjunction with findings from these previous studies suggest that doxycycline binds to plasma proteins in northern elephant seals at a rate intermediate between the rates for cats and horses. Alternatively, other factors such as intrinsic tear properties or transport mechanisms may play a more important role in aquatic mammals such as elephant seals, compared with terrestrial mammalian species. Although a complete understanding of ocular tear film dynamics in pinnipeds is lacking, pinnipeds appear to have a relatively small but highly active lacrimal gland[20,21] and to lack the lipid layer of the tear film,[l] with the mucin layer produced by the Harderian gland likely playing a more important role than in terrestrial species.[m] Our finding in the present study that doxycycline could be detected in the tear film at least 6 days after the most recent dose of doxycycline suggested that an anatomic drug reservoir was formed. Because doxycycline is a lipophilic drug, it is reported to accumulate in the sebum and lipid-rich secretions of the meibomian glands of humans.[3,22] Taken together, these data suggest that there may be a lipid portion of the tear film in northern elephant seals or that doxycycline may accumulate in the Harderian gland of northern elephant seals.

Doxycycline is a broad-spectrum, bacteriostatic tetracycline antibiotic likely to be effective against some of the most important pathogens in the marine environment, including *Brucella* spp, *Streptococcus* spp, *Leptospira* spp, *Staphylococcus* spp, and *Vibrio* spp.[6,23,24] The MIC of doxycycline for various bacteria (including human isolates) is 0.06 to 2 µg/mL for *Mycoplasma mycoides*,[25] 0.12 to 0.5 µg/mL for *Staphylococcus aureus*,[26,27] 0.1 µg/mL for *Bacillus anthracis*,[28] 0.016 to 0.5 µg/mL for *Streptococcus pneumoniae*,[29] and 0.008 to 0.031 µg/mL for *Chlamydia pecorum*.[30] Although doxycycline efficacy was not assessed in the present study, it can be estimated on the basis of the AUC:MIC ratio. As tetracyclines have both time-dependent and concentration-dependent pharmacodynamics, multiple studies[31,32] have determined that the AUC:MIC ratio is the best predictor of efficacy for tetracyclines. The calculated AUC:MIC ratio was > 100 for bacteria for which the MIC of doxycycline was ≤ 0.25 µg/mL for the 20 mg/kg dose on both days 1 and 4 and for the 10 mg/kg dose on day 1. An AUC:MIC ratio > 100 has been shown to be effective at treating susceptible infections.[31,33] This includes most bacteria susceptible to doxycycline; however, for less susceptible bacteria (MIC > 0.5 µg/mL), a higher dose or more frequent administration of doxycycline may be warranted. Doxycycline is also cited as the treatment of choice for *Vibrio* keratitis in humans, rickettsial diseases, and leptospirosis in veterinary patients.[6,24,34,35] Results of the present study suggested that administration of 10 or 20 mg of doxycycline/kg achieves an AUC:MIC ratio likely to be efficacious for susceptible organisms (including some *Chlamydia* spp, *Staphylococcus* spp, and *Mycoplasma* spp) after only 1 day of drug administration. We were unable to calculate AUC for tear doxycycline concentration because of the sample design. However, likely antimicrobial efficacy of doxycycline in tears can also be estimated on the basis of the time the tear doxy-

cycline concentration exceeds the MIC. Although tear doxycycline concentrations in the present study were only about 10% of those achieved in plasma, they exceeded the MIC for some bacteria during drug administration but declined to concentrations unlikely to have antimicrobial activity following cessation of treatment, even in animals in which doxycycline could be detected in tears for 6 days following cessation of treatment. Taken together, these plasma and tear data suggested that northern elephant seals should receive doxycycline PO at a dose of at least 10 mg/kg/d and that treatment should be continued until clinical resolution of ocular signs is observed.

In addition to its antimicrobial properties, doxycycline has been found to exert additional therapeutic effects of relevance to treatment of corneal disease. Doxycycline blocks interleukin-1 and thus matrix metalloproteinase-9 synthesis, reducing clinical signs associated with keratoconjunctivitis sicca, meibomitis, and infected and noninfected corneal ulcers; improves corneal smoothness; and permits corneal re-epithelialization.[36,37] An ability to reduce matrix metalloproteinase activity is especially important because these proteases promote collagenolysis and lead to corneal matrix degradation, corneal malacia, and potentially globe rupture. These effects are believed to occur at concentrations lower than those required for antimicrobial activity. For example, in a recent study,[36] once-daily oral administration of 100 mg of doxycycline to humans did not produce detectable tear doxycycline concentrations but did reduce the tear matrix metalloproteinase-9 concentrations that are responsible for disease progression. Although antiprotease and anti-inflammatory activity was not tested in that study,[36] multiple other studies[37,38] indicate that doxycycline, even at subantimicrobial concentrations, has important anti-inflammatory and anti–matrix metalloproteinase activity in people. Thus, it is likely that the doxycycline concentrations achieved in the tear film of northern elephant seals in the present study receiving 10 or 20 mg of doxycycline/kg would be expected to exert anti-inflammatory, antiprotease, or other nonmicrobial therapeutic activities.

As is often the case in drug studies conducted in wildlife species,[33] the present study used population pharmacokinetics to minimize the volume and number of blood samples collected from individual animals. Such studies minimize stress to individual animals, permit less frequent blood sampling, and allow for biological variability; however, they require that samples be collected from a larger number of animals. There are several approaches to analysis of population pharmacokinetic data, including the NPD and NAD methods used in the present study as well as nonlinear mixed effects modeling. The NPD method uses every sample but assumes they originated from 1 animal, whereas the NAD method calculates the mean concentration at each time point and models this as a single animal.[33] In the present study, pharmacokinetic values generated by the NPD and NAD approaches were similar for data generated following administration of a single dose of 10 or 20 mg of doxycycline/kg and following administration of multiple doses of 20 mg of doxycycline/kg. However,

there was some disparity between the values achieved with NPD and NAD approaches with respect to elimination half-life and subsequently Cl/F. This observation may be due to variation in sampling time because the NPD approach allows for each time point to be entered as the actual sampling time and then modeled as a single model. In contrast, the NAD approach requires that the mean of both sampling time and drug concentration be calculated. The biggest disadvantage of the NPD and NAD approaches is that measurements of variability cannot be calculated, thus making statistical analysis of results impossible. Nonlinear mixed-effects modeling allows for measurement of population variability but requires a larger number of animals and more frequent sampling than was performed in the present study.

Results of the present study may facilitate management of debilitating corneal and systemic diseases in northern elephant seals and related captive marine mammal species in zoos and aquariums, and the results may have broader application to treatment and stewardship of rehabilitated marine mammals being released back into wild populations.

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Effect of large colon ischemia and reperfusion on concentrations of calprotectin and other clinicopathologic variables in jugular and colonic venous blood in horses

Astrid Grosche et al

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Objective—To determine the effect of large colon ischemia and reperfusion on concentrations of the inflammatory neutrophilic protein calprotectin and other clinicopathologic variables in jugular and colonic venous blood in horses.

Animals—6 healthy horses.

Procedures—Horses were anesthetized, and ischemia was induced for 1 hour followed by 4 hours of reperfusion in a segment of the pelvic flexure of the large colon. Blood samples were obtained before anesthesia, before induction of ischemia, 1 hour after the start of ischemia, and 1, 2, and 4 hours after the start of reperfusion from jugular veins and veins of the segment of the large colon that underwent ischemia and reperfusion. A sandwich ELISA was developed for detection of equine calprotectin. Serum calprotectin concentrations and values of blood gas, hematologic, and biochemical analysis variables were determined.

Results—Large colon ischemia caused metabolic acidosis, a significant increase in lactate and potassium concentrations and creatine kinase activities, and a nonsignificant decrease in glucose concentrations in colonic venous blood samples. Values of these variables after reperfusion were similar to values before ischemia. Ischemia and reperfusion induced activation of an inflammatory response characterized by an increase in neutrophil cell turnover rate in jugular and colonic venous blood samples and calprotectin concentrations in colonic venous blood samples.

Conclusions and Clinical Relevance—Results of this study suggested that large colon ischemia and reperfusion caused local and systemic inflammation in horses. Serum calprotectin concentration may be useful as a marker of this inflammatory response. (*Am J Vet Res* 2013;74:1281–1290)

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