Naïve averaged, naïve pooled, and population pharmacokinetics of orally administered marbofloxacin in juvenile harbor seals

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Objective—To determine the pharmacokinetics of marbofloxacin after oral administration in juvenile harbor seals (*Phoca vitulina*) at a dose of 5 mg/kg (2.3 mg/lb) and to compare pharmacokinetic variables after pharmacokinetic analysis by naïve averaged, naïve pooled, and nonlinear mixed-effects modeling.

Design—Original study.

Animals—33 male and 22 female juvenile seals being treated for various conditions.

Procedures—Blood collection was limited to ≤ 3 samples/seal. Plasma marbofloxacin concentrations were measured via high-pressure liquid chromatography with UV detection.

Results—Mean ± SE dose of marbofloxacin administered was 5.3 ± 0.1 mg/kg (2.4 ± 0.05 mg/lb). The terminal half-life, volume of distribution (per bioavailability), and clearance (per bioavailability) were approximately 5 hours, approximately 1.4 L/kg, and approximately 3 mL/min/kg, respectively (values varied slightly with the method of calculation). Maximum plasma concentration and area under the plasma-time concentration curve were approximately 3 μg/mL and 30 h•μg/mL, respectively. Naïve averaged and naïve pooled analysis appeared to yield a better fit to the population, but nonlinear mixed-effects modeling yielded a better fit for individual seals.

Conclusions and Clinical Relevance—Values of pharmacokinetic variables were similar regardless of the analytic method used. Pharmacokinetic variability can be assessed with nonlinear mixed-effects modeling, but not with naïve averaged or naïve pooled analysis. Visual observation by experienced trainers revealed no adverse effects in treated seals. Plasma concentrations attained with a dosage of 5 mg/kg every 24 hours would be expected to be efficacious for treatment of infections caused by susceptible bacteria (excluding *Pseudomonas aeruginosa*). (*J Am Vet Med Assoc* 2007;230:390–395)

Marbofloxacin is a fluoroquinolone antimicrobial developed exclusively for veterinary use. It has a broad spectrum of activity that includes gram-positive and gram-negative aerobic bacteria and typically includes *Pseudomonas aeruginosa*.[1-3] Marbofloxacin has the highest activity of the veterinary fluoroquinolones against *P aeruginosa*.[2] The drug is well absorbed after oral administration and has a wide safety margin with few adverse effects in dogs.[3,4] Marbofloxacin pharmacokinetics have been determined in multiple veterinary species but, to the authors' knowledge, have not been reported for any marine mammals.[3-7]

Marbofloxacin is a concentration-dependent antimicrobial that has a postantibiotic effect. Targeted plasma concentrations for concentration-dependent antimicrobials are arrived at on the basis of bacterial MIC values. The MIC of marbofloxacin in Enterobacteriaceae, *Pasteurella multocida*, and *Staphylococcus* spp is typically ≤ 0.3 μg/mL. The MIC for susceptible *P aeruginosa* isolates is typically higher, at 1 μg/mL.[1] Ideal dosing regimens target the AUC in a 24-hour period ($AUC_{0\text{-}24}$) at 100 to 125 times the MIC for gram-negative bacteria, with a target of 30 h•μg/mL for non–*P aeruginosa* species.[8-10] Control of gram-positive bacteria may require a lower $AUC_{0\text{-}24}$/MIC ratio; values as low as 50 have been reported.[11]

Pharmacokinetic studies in domestic animals typically involve small groups of animals (usually 5 or 6) that undergo frequent collection of plasma samples. Pharmacokinetic variables are calculated and expressed as mean values with an associated SE or SD, a technique that is termed a standard 2-stage method. Disadvantages of the standard 2-stage method include the need for frequent sample collection and the fact that studies are conducted on a homogeneous, usually healthy, population and results may not represent the variable

Abbreviations

MIC	Minimum inhibitory concentration
AUC	Area under the plasma concentration-time curve

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estimates for the target population. Also, standard 2-stage models cannot be used to determine the source of variation (interindividual vs intraindividual) because the variation is grouped into an SE or SD value.[12]

Population pharmacokinetic studies typically include large numbers of animals of the target population, from which samples are infrequently obtained. The population is typically more diverse than those evaluated in standard 2-stage studies and allows for estimation of biological variability. Population pharmacokinetic modeling also estimates inter- and intraindividual sources of variability independently. Nonlinear mixed-effects modeling is a parametric analytic method that estimates fixed effects (θ), random effects (η), and covariates. The fixed effects are variables that do not vary randomly among individuals and are usually representative of typical values for a population (ie, volume of distribution and absorption rate). In contrast, random effects do vary randomly among individuals within a population and are a source of interindividual variation. Covariates may also be included in population pharmacokinetic models and represent either a discrete (eg, male or female) or continuous dependent (eg, creatinine concentration) variable with a functional relationship to the independent variable θ and may represent inter- or intraindividual variability, depending on the variable. For example, sex is an example of interindividual variability, whereas concurrent drug administration can be an intraindividual variable.[12] Population pharmacokinetic modeling (nonlinear mixed-effects modeling) has been used to describe the pharmacokinetics of several drugs in veterinary species.[13-16]

Naïve averaged and naïve pooled pharmacokinetic methods can also be used to assess a population of animals from which samples are infrequently obtained. Naïve averaged pharmacokinetic analysis involves calculating the mean concentration at each time point and modeling the mean concentration as a single animal. Although this method can be used to calculate pharmacokinetic variables, it does not yield an estimate of population variability. Naïve pooled pharmacokinetic analysis involves modeling every sample, but the pharmacokinetic analysis treats all samples as if they originated from 1 animal.

In a previous study,[14] pharmacokinetic variables for oxytetracycline after IM administration in sheep and calves were investigated with multiple methods of pharmacokinetic analysis. In that study, standard 2-stage, naïve pooled, and population pharmacokinetic (nonlinear mixed-effects) modeling was used, and results were similar regardless of the method of analysis. However, a large number of samples ($\geq$ 12) were collected from each animal.

Sampling wild or captive animals is challenging because of the difficulties in restraining wildlife species and the stress induced in the animals. Therefore, population pharmacokinetic analysis is a potentially useful approach for obtaining data from a target population of wild animals. A population pharmacokinetic approach enables an investigator to collect few samples from a large group of animals, thereby minimizing the problems associated with more frequent sampling. Population pharmacokinetic analysis may be conducted with naïve averaged, naïve pooled, or nonlinear mixed-effects modeling approaches. However, comparison of these techniques in veterinary studies in which a sparse sampling protocol was used has not been published, to the authors' knowledge.

The purposes of this study were to evaluate the plasma profile of marbofloxacin after 5 mg/kg doses were administered orally to harbor seals and compare calculated pharmacokinetic variables after naïve averaged, naïve pooled, and nonlinear mixed-effects modeling.

Materials and Methods

Animals—Harbor seals that were brought to the Vancouver Aquarium Marine Science Centre for rehabilitation from 2002 to 2003 were included in the study. The study was performed in compliance with Vancouver Aquarium Marine Science Centre guidelines. Marbofloxacin[a] was administered as 25- or 50-mg tablets, with a targeted dose of 5 mg/kg, to the nearest half tablet. The drug was administered via orogastric intubation with fish slurry or was placed in fish and fed directly for 5 to 10 days. Blood samples were collected after the first dose by venipuncture from the caudal venous sinus and immediately placed in glass tubes containing lithium heparin. Plasma was separated and stored frozen at –20°C until analysis. No more than 3 plasma samples were collected from any seal. The timing of sample collections was predetermined to obtain an orthogonal sampling design (**Table 1**). Seals were observed at least twice daily by experienced rehabilitators for signs of adverse effects.

Plasma drug analysis—Plasma drug concentrations were measured via high-pressure liquid chromatography with UV detection after solid-phase extraction, according to a previously published method.[17] Seal plasma was fortified with marbofloxacin to construct daily standard curves at concentrations of 0, 0.05, 0.1, 1, 2, and 5 µg/mL. The standard curves were accepted if the coefficient of determination (r^2) was > 0.99 and measured concentrations were within 15% of actual concentrations. Plasma concentrations > 5 µg/mL were diluted with untreated seal plasma prior to analysis.

Pharmacokinetic analysis—Naïve averaged pharmacokinetic variables were calculated from the mean plasma concentration at each time point with computer software.[b] Plasma concentrations were normalized to a concentration of 5 mg/kg by multiplying the measured plasma concentration by 5 mg/kg and dividing by the actual dose administered (in mg/kg). Pharmacokinetic variables were calculated from equations published elsewhere.[18] The pharmacokinetic model selected was a 1-compartment model with first-order input and first-order output. The model was selected on the basis of residual plots and visual examination of actual and predicted data. The formula for the model was as follows:

$$C_t = \frac{D \cdot K_a}{(V/F) \cdot (K_a - K_{el})} \cdot e^{-K_{el} \cdot t} - e^{-K_a \cdot t}$$

where C_t is the plasma concentration at time t; D is the dose (mg/kg); K_a and K_{el} are the absorption rate and elimination rate constants (h^{-1}), respectively; V/F is the apparent volume of distribution per bioavailability (L/kg);

Table 1—Example of the template used to schedule blood collection in 12 harbor seals that received marbofloxacin (5 mg/kg [2.3 mg/lb], PO) in the first year (2002) of a 2-year study of marbofloxacin pharmacokinetics. The template used in 2003 was the same except that the 24-hour time point was changed to 20 hours.

	Time after administration (h)								
Seal	0.25	0.5	0.75	1	2	4	8	16	24
1	X			X				X	
2	X			X					X
3	X					X	X		
4	X				X	X			
5		X		X					X
6		X		X				X	
7		X			X		X		
8		X			X			X	
9			X			X	X		
10			X		X				X
11			X			X		X	
12			X				X		X

e is the base of the natural logarithm; and t is time (h). The AUC was calculated by use of the linear trapezoidal rule for all 3 methods of analysis. The total body clearance per bioavailability (Cl/F), absorption half-life ($T_{1/2}$ K_a), terminal half-life ($T_{1/2}$ K_{el}), time to maximal plasma concentration (T_{max}), and maximal plasma concentration (C_{max}) were also calculated.

Naïve pooled pharmacokinetic analysis was performed in a similar manner with the same computer program, but all of the measured plasma concentrations normalized to a dose of 5 mg/kg were input. The pharmacokinetic variables were calculated in a manner similar to that used for the naïve averaged analysis.

Nonlinear mixed-effects analysis was performed with a computer program[c] by use of actual dosages administered and actual measured plasma concentrations. The pharmacokinetic model and variables selected were as for the naïve averaged and naïve pooled analysis. A normal distribution of the random effects was assumed. The parameters calculated for the nonlinear mixed-effects model were $V/F = \theta_1 \cdot e^{\eta 1}$, $K_a = \theta_2 \cdot e^{\eta 2}$, $K_{el} = \theta_3 \cdot e^{\eta 3}$, where θ is the associated fixed effect and η is the associated random effect. Because of the homogeneous aspect of the population, covariates were not assessed.

Results

Fifty-five seals were included in the study. Of the 31 harbor seals that were enrolled in the study in 2002, 18 were males and 13 were females, with an age range of 1 to 45 days and weights ranging from 6.2 to 13.4 kg (13.6 to 29.5 lb). Of the 24 harbor seals enrolled in 2003, 15 were males and 9 were females, with an age range of 16 to 92 days and weights ranging from 6.0 to 33.0 kg (13.2 to 72.6 lb). Mean ± SE dose of marbofloxacin administered was 5.3 ± 0.1 mg/kg. On the basis of visual inspections by experienced rehabilitators, no adverse effects of treatment were observed in treated seals.

Measured plasma marbofloxacin concentrations were in a range that was anticipated on the basis of results in other animals (**Table 2**). The calculated pharmacokinetic variables and predicted plasma profiles for the naïve averaged, naïve pooled, and nonlinear mixed-effects modeling were similar (**Figure 1**; **Table 3**).

Table 2—Mean marbofloxacin plasma concentrations in 55 harbor seals after oral administration of the drug at a mean ± SE dose of 5.3 ± 0.1 mg/kg.

Time after administration (h)	Mean plasma concentration (μg/mL)	SE
0.25	1.55	0.28
0.5	1.84	0.28
0.75	3.16	0.44
1	2.60	0.31
2	2.94	0.20
4	2.28	0.23
6	2.25	0.29
8	0.93	0.23
16	0.65	0.14
20	0.28	0.07
24	0.40	0.08

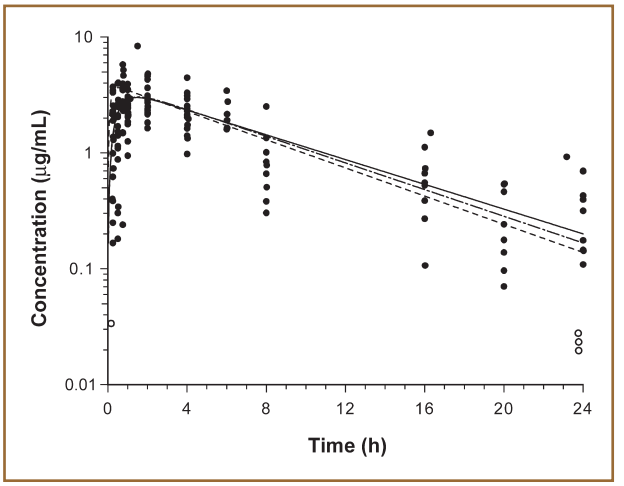

Figure 1—Plasma concentrations of marbofloxacin normalized to a concentration of 5 mg/kg PO during the first 24 hours after oral administration to 55 harbor seals. Closed circles = Plasma concentration. Open circles = Plasma concentration was below the limit of quantification. Solid line = Values predicted on the basis of naïve averaged pharmacokinetic analysis. Dotted-and-dashed line = Values predicted on the basis of naïve pooled pharmacokinetic analysis. Dotted line = Values predicted on the basis of nonlinear mixed-effects pharmacokinetic analysis.

Table 3—Predicted values for pharmacokinetic variables after naïve averaged, naïve pooled, and nonlinear mixed-effects pharmacokinetic modeling in the same 55 seals as in Table 2.

Variable	Units	Naïve averaged	Naïve pooled	Nonlinear mixed-effects		
				Fixed effect	Mean	SE
K_a	h^{-1}	2.03	1.83	2.00	8.86	2.44
K_{el}	h^{-1}	0.12	0.13	0.15	0.20	0.02
V/F	L/kg	1.39	1.34	1.53	1.29	0.08
AUC	h•μg/mL	29.23	28.12	NA	30.91	2.05
$T_{1/2}$ K_a	h	0.34	0.38	NA	0.66	0.13
$T_{1/2}$ K_{el}	h	5.64	5.23	NA	4.96	0.38
Cl/F	mL/min/kg	2.85	2.96	NA	3.64	0.32
T_{max}	h	1.47	1.55	NA	1.54	0.19
C_{max}	μg/mL	3.00	3.03	NA	3.44	0.15

K_a = Absorption rate constant. K_{el} = Elimination rate constant. V/F = Apparent volume of distribution per bioavailability. $T_{1/2}$ K_a = Absorption half-life. $T_{1/2}$ K_{el} = Terminal half-life. Cl/F = Total body clearance per bioavailability. T_{max} = Time to maximal plasma concentration. C_{max} = Maximal plasma concentration. NA = Not applicable.

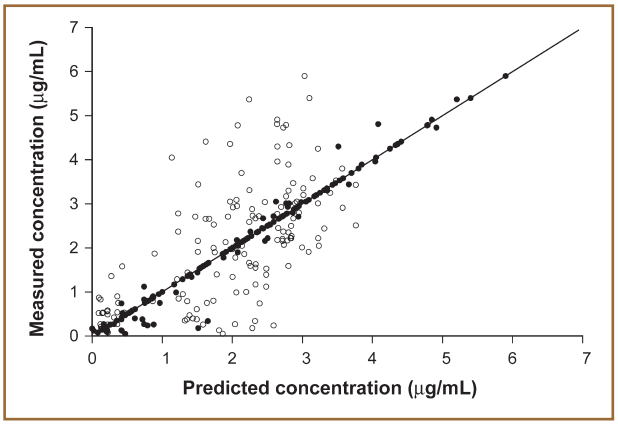

Figure 2—Correlation between measured plasma marbofloxacin concentrations and concentrations predicted with nonlinear mixed-effects modeling after oral administration of the drug (mean dose, 5.3 ± 0.1 mg/kg) in 50 harbor seals. Closed circles = Predicted individual values (with inclusion of η; r^2 = 0.98). Open circles = Predicted population values (with inclusion of θ only; r^2 = 0.42). Solid line = Line of unity.

Compared with the naïve averaged and naïve pooled methods, nonlinear mixed-effects modeling estimated a similar AUC, more rapid elimination rate, and higher estimated clearance (per bioavailability; Figure 1; Table 3). The naïve averaged and naïve pooled methods appeared to predict the concentrations at the later time points more accurately, but 3 samples at 24 hours were excluded from pharmacokinetic analyses because they were below the limit of quantification of the assay. The nonlinear mixed-effects model appeared to underpredict the 24-hour time-point value; however, if the time points at which values were below the limit of quantification are taken into account, the estimated concentrations may be more accurate.

The nonlinear mixed-effects modeling predicted individual seals' plasma concentration by inclusion of the fixed (θ) and random effects (η), with a high coefficient of determination (r^2 = 0.98; **Figure 2**). The correlation, without inclusion of the random effects (population predicted), was r^2 = 0.42 for the nonlinear mixed-effects modeling. However, individual pharmacokinetic variables from 5 seals were excluded from the nonlinear mixed-effects modeling because the calculated values were not accurate. For example, the elimination half-life for one of the seals was calculated as 69,314,718.06 hours.

Discussion

Calculated pharmacokinetic variables were similar whether naïve averaged, naïve pooled, or population pharmacokinetic analyses were used to model the disposition of marbofloxacin in juvenile harbor seals. These findings are similar to those from an earlier study[15] in which the pharmacokinetics of oxytetracycline in sheep and calves were determined by standard 2-stage, naïve pooled, and nonlinear mixed-effects modeling. In contrast to the present study, a larger number of samples (at least 12) were collected from each animal in the earlier study.[15]

Naïve averaged and pooled analyses of marbofloxacin yielded similar calculated pharmacokinetic estimates for the population. As expected, the nonlinear mixed-effects modeling approach was a better fit for individual seals; however, it is important to examine the data generated for each seal because certain individual predicted values appeared to be inaccurate (ie, $T_{1/2}$ K_{el} = 69,314,718.06 hours). These findings suggest that investigators may select an analysis that best suits their sampling capability and study population when designing future studies. When it is not possible to collect samples at consistent time intervals, naïve pooled analysis and nonlinear mixed-effects modeling may be used to estimate pharmacokinetic variables in a population. When a measure of population variability is required, a nonlinear mixed-effects model may be more appropriate.

Marbofloxacin appeared to be well tolerated in the treated seals. Because the drug was administered for < 10 days, long-term effects could not be evaluated. Cartilage damage was detected in 16 juvenile dogs that were examined after 14 days of high-dose (11 mg/kg/d [5 mg/lb/d]) marbofloxacin treatment (New Animal Drug Application 141-151). Lameness was noticed in all dogs by the third day of treatment, with additional clinical signs of hunched posture, limited use of hind limbs, ataxia, decreased activity levels, decreased appetite, and decreased fecal output. No similar clinical signs were observed in the harbor seals; however, that does not necessarily preclude the absence of articular cartilage damage. Examination of articular cartilage was not performed in the present study because all treated seals survived.

Although the present study was not designed to assess efficacy, plasma concentrations achieved were high

enough to yield AUC/MIC ratios that have been associated with successful treatment of infections in other animals.[9-11] Values of AUC measured from our analyses were high enough to yield an AUC/MIC ratio > 100 for bacteria with MIC values < 0.25 µg/mL. However, infections caused by less susceptible bacteria (eg, *P aeruginosa*) with a higher MIC value (eg, 1 µg/mL) may require higher doses of marbofloxacin than were used in the present study.

The calculated volume of distribution (per bioavailability) of marbofloxacin in the harbor seals was similar (approx 1.4 L/kg) to the volume of distribution in adult dogs (1.4 ± 0.2 L/kg),[3] a similar-sized mammal. Although pharmacokinetic analysis after IV administration of the drug would be necessary to determine the true volume of distribution (or clearance or bioavailability), this was not undertaken because every effort was made to minimize stress to the seals given their health conditions and the fact that they are a wildlife species.

Although values for C_{max} were similar between dogs and seals when the dose was normalized to 5 mg/kg, the terminal half-life of marbofloxacin in harbor seals (approx 5 hours) was shorter than that observed in dogs after oral administration (12.5 ± 2.7 hours). As expected, the calculated clearance per bioavailability of marbofloxacin was higher in harbor seals (approx 3 mL/min/kg), compared with the actual clearance in dogs (1.67 ± 0.4 mL/min/kg).[3] In dogs, the major routes of elimination after oral administration are the feces (unchanged drug) and urine (accounting for approx 40% of drug elimination).[3,19] The mechanism of elimination in seals was not evaluated. Differences in the terminal half-life of marbofloxacin between dogs and seals may be caused by factors that were not measured in this study (eg, true clearance and volume of distribution) involving oral drug administration.

Values of pharmacokinetic variables calculated by naïve averaged and naïve pooled analysis were similar. This similarity was not surprising because both methods depend on estimating a curve to best fit the averaged plasma concentrations, and the pharmacokinetic analysis was similar. Naïve averaged analysis requires calculation of the mean plasma concentration at each time point and modeling of that value as a single animal to estimate the pharmacokinetic variables, whereas naïve pooled analysis treats all samples as if they came from a single animal. A disadvantage of naïve averaged analysis in a field study is that plasma samples cannot always be obtained at precise sampling times to obtain a mean concentration at each point. Therefore, the present study was planned with the objective of comparing other methods of obtaining population pharmacokinetic variables (naïve pooled and nonlinear mixed-effects modeling) that allow for more flexible sampling schemes. Naïve pooled analysis allows each time point to be entered as the actual time taken and then modeled to a single model. Therefore, naïve pooled analysis would be expected to decrease error and increase accuracy of the model, compared with naïve averaged analysis. Both naïve averaged and naïve pooled analysis require normalization of plasma concentrations to a common dose, which could result in inaccurate analysis if drug pharmacokinetics are not linear (that is, if AUC and C_{max} are not proportional to dose). However, if the dose range is small as it was in the present study (5.3 ± 0.1 mg/kg), less error from dose normalization would be expected. Marbofloxacin has linear (dose independent) pharmacokinetics in other species, so error resulting from normalization of dosages is expected to be small.

Population pharmacokinetics enables assessment of covariates (pathophysiologic variables), which could induce alterations in drug absorption, distribution, metabolism, and elimination. As confirmed by the high r^2 value (0.98) for predicted and measured plasma concentrations with nonlinear mixed-effects modeling, the model was accurate without the inclusion of covariates. The population assessed was homogeneous, with all seals being a similar age and having plasma concentrations of creatinine, urea nitrogen, albumin, total bilirubin, total protein, and plasma alkaline phosphatase activity within expected ranges for the population. The uniformity of the study population is likely the reason why individual predicted values correlated well with measured values. It is also possible that the pharmacokinetics of marbofloxacin are minimally affected by concurrent disease and that this also contributed to the homogeneous results; in an earlier study in dogs,[19] renal disease had minimal effects on marbofloxacin disposition in dogs with abnormal clinical chemistry values and glomerular filtration rate.

Pharmacokinetic analysis of marbofloxacin with a sparse sampling protocol yielded values for variables that were similar whether naïve averaged, naïve pooled, or nonlinear mixed-effects (population pharmacokinetic) modeling was used. Naïve averaged and pooled analyses of marbofloxacin yielded similar pharmacokinetic estimates for the population. Population pharmacokinetics resulted in a better individual fit for individual seals. Marbofloxacin was well tolerated when administered orally at 5 mg/kg to harbor seals and achieved a plasma profile and concentrations that would be expected to be effective in treatment of susceptible bacterial infections.

a. Zeniquin, Pfizer Animal Health, New York, NY.
b. WinNonlin, version 4.0.1, Pharsight Corp, Mountain View, Calif.
c. WinNonMix, version 2.0.1, Pharsight Corp, Mountain View, Calif.

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