Comparison of plasma and interstitial fluid concentrations of doxycycline and meropenem following constant rate intravenous infusion in dogs

Tara L. Bidgood, DVM, and Mark G. Papich, DVM, MS

Objective—To compare plasma (total and unbound) and interstitial fluid (ISF) concentrations of doxycycline and meropenem in dogs following constant rate IV infusion of each drug.

Animals—6 adult Beagles.

Procedure—Dogs were given a loading dose of doxycycline and meropenem followed by a constant rate IV infusion of each drug to maintain an 8-hour steady state concentration. Interstitial fluid was collected with an ultrafiltration device. Plasma and ISF were analyzed by high performance liquid chromatography. Protein binding and lipophilicity were determined. Plasma data were analyzed by use of compartmental methods.

Results—Compared with meropenem, doxycycline had higher protein binding (11.87% [previously published value] vs 91.75 ± 0.63%) and lipophilicity (partition coefficients, 0.02 ± 0.01 vs 0.68 ± 0.05). A significant difference was found between ISF and plasma total doxycycline concentrations. No significant difference was found between ISF and plasma unbound doxycycline concentrations. Concentrations of meropenem in ISF and plasma (total and unbound) were similar. Plasma half-life, volume of distribution, and clearance were 4.56 ± 0.57 hours, 0.65 ± 0.82 L/kg, and 1.66 ± 2.21 mL/min/kg, respectively, for doxycycline and 0.73 ± 0.07 hours, 0.34 ± 0.06 L/kg, and 5.65 ± 2.76 mL/min/kg, respectively, for meropenem. The ISF half-life of doxycycline and meropenem was 4.94 ± 0.67 and 2.31 ± 0.36 hours, respectively.

Conclusions and Clinical Relevance—The extent of protein binding determines distribution of doxycycline and meropenem into ISF. As a result of high protein binding, ISF doxycycline concentrations are lower than plasma total doxycycline concentrations. Concentrations of meropenem in ISF can be predicted from plasma total meropenem concentrations. (*Am J Vet Res* 2003;64:1040–1046)

Successful antimicrobial therapy is dependent on achieving adequate drug concentrations at the site of infection for a sufficient duration, the susceptibility of the organism to the antimicrobial, pharmacokinetic parameters of the drug, and the local environment. The ultimate goal of antimicrobial therapy is to obtain a clinical and bacteriologic cure without causing toxic effects or the development of resistant bacteria.[1] Most bacterial infections are in the extracellular space of tissues; therefore, to achieve therapeutic success, antimicrobial agents must be able to distribute adequately to this region.[2] The distribution of most drugs in the body obey Fick's law of diffusion in which the rate of movement (flux) is dependent on the concentration gradient, diffusion coefficient, partition coefficient, and thickness of the membrane. Because this is a linear or first order process, the rate of drug movement is dependent on the dose. Only small, unbound, nonionized, and lipophilic molecules can diffuse across most membranes. For drugs that are protein bound, the total concentration (bound and unbound) is higher in plasma than in tissue fluid (ie, **interstitial fluid [ISF]**), but the unbound drug equilibrium across the capillary membrane produces equal amounts of free (unbound) drug concentrations in both compartments.[3] Because only the unbound drug is microbiologically active, determining the concentrations of unbound drug at the active site is important for the evaluation of clinical efficacy.[4]

Therapeutic drug monitoring and plasma **minimum inhibitory concentrations (MICs)** do not account for the drug fraction bound to proteins; in addition, the total drug concentrations are usually measured in pharmacokinetic studies to predict therapeutic activity. Use of plasma total antimicrobial concentrations for predicting pharmacokinetic-pharmacodynamic relationships for some drugs may be misleading and could result in overestimation of therapeutic efficacy.[5] Low unbound drug concentrations at the site of action could result in a subtherapeutic effect and increase the risk for the development of antimicrobial resistance.[5]

For drugs with low plasma protein binding, the plasma total drug concentration is an accurate representation of the drug concentration at the site of action in the ISF; we have demonstrated this in a single-dose study with a low protein-bound β-lactam.[6] For highly protein-bound drugs, the plasma total drug concentration, without accounting for the degree of protein binding, may result in an inaccurate assessment of the drug concentration at the site of action (ie, in the ISF).[3] Measuring the unbound concentration of these drugs at the target site (ie, in the ISF) may be a better predictor of therapeutic efficacy than plasma concentrations.[5] To achieve a better understanding of the dynamics of drug distribution, we chose to evaluate doxycycline and meropenem because they differ in their physiochemical properties, pharmacokinetic parameters, and protein binding.

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From the Department of Molecular Biomedical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, NC 27606.
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Address correspondence to Dr. Bidgood.

Doxycycline is a second-generation tetracycline derivative, and its advantages stem from having a broad spectrum of activity, formulations for oral and IV administration, limited adverse effects, good oral absorption, and being relatively inexpensive. It is a broad spectrum antimicrobial with activity against gram-negative, gram-positive, and anaerobic bacteria and many intracellular organisms.[7,8] Doxycycline's high lipophilicity is responsible for better fluid and tissue penetration, longer **half-life** ($t_{1/2}$), increased oral absorption, and enhanced in vitro antimicrobial activity, compared with the first generation tetracyclines.[9,10] Doxycycline is extensively protein bound in most species.[9,11-14] Rapid administration of doxycycline has been associated with adverse effects in some species and is thought to be the result of the chelation of calcium.[15]

Meropenem is a member of the carbapenem family of β-lactam antimicrobials, and the pharmacokinetics and ISF concentrations from a single IV and SC administration have recently been published.[6] Its plasma protein binding was 11.87% in that study.[6]

With a single bolus administration of drugs by any route, a lag time for drug distribution to the target site exists, making the comparison between plasma and ISF concentrations difficult. Therefore, to accurately evaluate the dynamics of drug distribution to tissues, steady state plasma concentrations are necessary by administering a **constant rate IV infusion (CRI)**. Equilibrium between plasma and ISF can then be maintained during the IV infusion period, and samples from each compartment can be collected, analyzed, and compared pharmacokinetically. By administering a CRI, the variability and influence of the $t_{1/2}$ can be eliminated, thereby allowing for a comparison between the 2 drugs.

In a previous study,[6] we showed that an in vivo ultrafiltration device could be used to collect ISF, allowing an evaluation of the relationship between drug concentrations in plasma and ISF. The in vivo ultrafiltration device provided advantages over the use of tissue biopsy specimens or tissue cages in the measurement of ISF drug concentrations in animals. Advantages of the ultrafiltration device are that SC implantation of the semipermeable probes is minimally invasive and it allows continuous collection of protein-free ISF from conscious animals. The purpose of the study reported here was to evaluate plasma (total and unbound) and ISF concentrations of 2 antimicrobials that differ in the extent of protein binding and lipophilicity and determine the effect that each of these characteristics has on the distribution between plasma and the target site (ie, the ISF) at steady state.

Materials and Methods

Animals—Six (3 male and 3 female) healthy adult Beagles (body weight, 6 to 9 kg) were used in this study. The study was reviewed and approved by the Institutional Animal Care Use Committee at North Carolina State University. Each of the dogs was determined to be clinically normal prior to the start of the study on the basis of physical examination findings and results of a CBC determination and serum biochemical analysis.

Procedure—A random crossover design approach was used in which the dogs were randomly assigned to 2 groups of 3. All dogs received both treatments. A 7-day washout period was provided between treatments. Doxycycline[a] and meropenem[b] were prepared as solutions (10 mg/mL) with sterile water the morning of the study. A loading dose of doxycycline (1.01 mg/kg) or meropenem (0.37 mg/kg) was administered immediately before initiating the 8-hour CRI (0.10 and 0.38 mg/kg/h for doxycycline and meropenem, respectively). The loading dose for doxycycline was administered for 5 minutes, and the loading dose for meropenem was given as a bolus injection. The CRI was administered by use of a programmed electronic infusion pump[c] to maintain a target concentration of 1 mg/mL during the 8-hour period. The CRI and loading dose were calculated from previously published pharmacokinetic values for dogs[6,12] by use of established equations.[16]

Collection of blood samples—Two jugular catheters were placed in each dog, 1 for blood collection and 1 for administration of the loading dose and CRI. Catheters were placed 18 hours prior to drug administration and flushed with sterile saline (0.9% NaCl) solution between collection times. Blood was collected from the jugular vein in evacuated glass tubes, with sodium heparin as the anticoagulant. Sample collection times were at 0 (pretreatment time), 10, 20, and 40 minutes and 1, 2, 4, 6, 8, 12, 16, 20, 24, and 28 hours during and after the CRI for both drugs. Blood was centrifuged after collection at 1,000 × *g* for 10 minutes, and plasma was separated and stored at –70°C until analysis.

ISF collection—Interstitial fluid collection was performed with an in vivo ultrafiltration device.[d] The 3 semipermeable loops of the ultrafiltration probe were inserted SC into the interstitial space with a guide needle, and the external tubing was connected to a 3-mL evacuated glass tube for collection of ISF. Lidocaine (0.3 mL) was injected at the insertion point of the guide needle. A new probe at a separate site was used for each drug and administration route. Ultrafiltration probes were placed 18 hours prior to the start of the study to allow fluid in the interstitial space and ultrafiltration probes to reach equilibrium. The membrane in the loops consisted of pores allowing water, electrolytes, and low molecular weight molecules (< 30,000 d) to pass. The evacuated glass tube provided the negative pressure for collection of ISF. The ISF was collected at 0 (pretreatment time), 2, 4, 6, 8, 12, 16, 20, 24, and 28 hours during and after the CRI. Interstitial fluid was collected and combined from 2 ultrafiltration probes in order to have sufficient volume for analysis. The fluid was immediately frozen at –70°C until analysis. Ultrafiltration probes were removed after the last collection time.

Protein binding—Plasma protein binding of meropenem was determined in a previous study[6] by the same method described here. Determination of plasma protein binding of doxycycline was performed with a micropartition device.[e] Three replicate concentrations (0.5, 1, and 2 µg/mL) were prepared by spiking pooled canine plasma with an analytical reference standard[f] and incubating them in a 37°C water bath for 30 minutes. One milliliter from each replicate was placed into a micropartition device reservoir. Devices were centrifuged for 30 minutes at 1,500 × *g*. Approximately 300 µL of ultrafiltrate was recovered in the filtrate cup. The doxycycline ultrafiltrate was processed and extracted as described for plasma prior to analysis by use of **high performance liquid chromatography (HPLC)**. Resulting concentrations represent the unbound fraction. A second set of 3 spiked replicates of the same concentrations was analyzed (300 µL), omitting the micropartition step. Resulting concentrations represent the total (bound and unbound) fraction. Concentrations were determined from a standard curve prepared with 300 µL of spiked plasma concentrations.

Percentage of the bound fraction was calculated by use of the following equation:

$$\%\ \text{protein binding} = \frac{\text{total concentration-unbound}}{\text{total concentration}} \times 100$$

Drug lipophilicity—To determine the lipid solubility of the 2 drugs, the octanol:water coefficient was determined by use of established methods.[17,18] A 0.1M sodium diphosphate buffer solution (aqueous phase) was prepared by use of sodium diphosphate[g] and deionized water. The pH was adjusted to 7.4 by adding a small amount of phosphoric acid.[h] Five milliliters of this aqueous buffer solution was spiked with meropenem or doxycycline (10 µg/mL) and added to an equal volume of octanol[i] in a screw top tube. The tube was gently rocked for 1 hour at room temperature (approx 25°C) to equally disperse the drug into each phase of the tube. The tube was then centrifuged for 10 minutes at 2,000 × *g* for phase separation. The aqueous layer was analyzed by HPLC without extraction before and after incubation and shaking. A calibration curve prepared from the spiked aqueous phase was used to determine the concentrations. The apparent partition coefficient was calculated by use of the following equation:

$$PC = \frac{\text{buffer concentration before incubation} - \text{buffer concentration after incubation}}{\text{buffer consentration after incubation}} \times \frac{\text{volume of buffer}}{\text{volume of octanol}}$$

Analysis—Plasma and ISF were analyzed by reverse phase HPLC with **ultraviolet (UV)** detection to determine the concentrations of meropenem and doxycycline. This system included 2 pumps,[j] a pump controller,[k] an automated sampler,[l] and a UV light detector. For meropenem, a 4.6 × 150-mm reverse phase column[m] was used for the separation. Solid phase extraction[n] was used to prepare plasma samples followed by reverse phase chromatography with UV detection at 296 nm. The mobile phase consisted of 85% 0.01M acetate buffer and 15% methanol at a pH of 4.3 and a flow rate of 1 mL/min. For doxycycline, plasma samples were prepared by first adding a releasing agent (78% water, 20% acetonitrile, 2% phosphoric acid) and vortexing the mixture. The mixture was then transferred into centrifugal filter units[o] for extraction. Filter units were centrifuged at 10,000 × *g* for 30 minutes. The supernatant was then analyzed by HPLC with UV detection at 350 nm by use of a 4.6 × 150-mm reverse phase column.[p] The ISF samples and fluid from the lipophilicity study for both drugs were analyzed by HPLC without extraction. Pure reference standards of each drug[f,q] were used to prepare calibration and quality control samples. Pooled blank canine plasma and PBS solution were used for the plasma and ISF calibration curves, as well as validation of the assay.

Pharmacokinetic analysis—A computer software program[r] was used to estimate the plasma and ISF pharmacokinetic parameters. Plasma data were analyzed by compartmental methods, and the model that best fit the data was determined by use of the minimal Aikake information criterion estimation method.[19] A 2 compartmental model with input for an IV bolus was used to analyze the plasma data for IV administration of doxycycline. A weighting factor of the reciprocal of the predicted concentrations squared was used. A continuous rate infusion noncompartmental model was used to analyze the ISF data for both drugs and plasma data for meropenem. For doxycycline, **clearance from the central compartment (Cl)**, **elimination $t_{1/2}$ ($t_{1/2\beta}$)**, **distribution $t_{1/2}$ ($t_{1/2\alpha}$)**, **apparent volume of distribution at steady state (Vd_{ss})**, microconstants (ie, elimination rate from compartment 1 [K10]; rate of movement from compartment 1 to compartment 2 [K12]; rate of movement from compartment 2 to compartment 1 [K21]), intercept for the distribution phase (A), intercept for the elimination phase (B), rate constant associated with the distribution phase (α), rate constant associated with the elimination phase (β), and **area under the curve (AUC)** were calculated for the compartmental model by use of published equations.[16] The ISF $t_{1/2}$ was calculated with the following formula:

$$t1/2 = 0.693/\lambda_z$$

where λ_z is the rate constant associated with the terminal portion of the curve.

The **mean residence time (MRT)**, which is the mean time that all molecules in a given dose spend in the body, was calculated with the following formula:

$$MRT = AUMC_{0\rightarrow\infty}/AUC_{0\rightarrow\infty}$$

where $AUC_{0\rightarrow\infty}$ is the AUC from time 0 to infinity and $AUMC_{0\rightarrow\infty}$ is the area under the moment curve from time 0 to infinity. The AUC for ISF was estimated with the log-linear trapezoidal rule and extrapolated to infinity by use of CT/λ_z, where CT is the last measured time concentration value.

Because of the limited data points during elimination, the AUC for CRI plasma meropenem concentration was calculated by determining the **partial AUC (AUC_{par})** between 1 and 8 hours during the steady state period by use of the trapezoidal method. The Cl and Vd_{ss} of meropenem after the CRI were then calculated with the following formulas:

$$Cl = dose/AUC_{par}$$
$$Vd = 1.44R_o t_{1/2}/C_{ss}$$

where R_o is the infusion rate and C_{ss} is the mean concentration at steady state. The **maximum concentration (C_{max})** and the **time to maximum concentration (T_{max})** were determined directly from the concentration versus time curves for ISF.

Statistical analysis—Plasma unbound and total concentrations of doxycycline and meropenem were compared with ISF concentrations of doxycycline and meropenem by use of mixed-effect models with different correlation structures.[s] By comparing the Aikake information criterion and Schwarz bayesian criterion values, the mixed model with a 1-dependent correlation structure was selected.

Results

Pharmacokinetic parameters for doxycycline and meropenem were estimated (**Table 1 and 2**). Because of the short $t_{1/2}$ and **limit of detection (LOD)**, the last detectable plasma concentration of meropenem was at 12 hours. Therefore, we were only able to estimate the plasma $t_{1/2}$ for meropenem from 8- and 12-hour plasma concentrations.

The **limit of quantification (LOQ)** and LOD in plasma after CRI were 0.025 and < 0.010 µg/mL, respectively, for doxycycline and 0.016 and < 0.010 µg/mL, respectively, for meropenem. The LOQ and LOD in ISF after CRI were 0.014 and < 0.010 µg/mL, respectively, for both drugs. Only values greater than the LOQ were used in the pharmacokinetic analysis.

Plasma (total and unbound) and ISF concentrations of doxycycline and meropenem after CRI were plotted against time (**Fig 1 and 2**). Because plasma unbound concentrations of doxycycline and meropenem were determined mathematically, by taking into account the extent of protein binding (91.75 ± 0.63% for doxycycline and 11.87%[6] for meropenem) of the total concentrations and not through direct measurement, no SDs for these mean values were included in

Table 1—Mean (± SD) values for pharmacokinetic parameters of doxycycline after constant rate IV infusion (0.10 mg/kg/h) in 6 dogs

Parameters	Plasma	Interstitial fluid
$AUC_{0\rightarrow\infty}$ (h•µg/mL)	12.09 ± 3.22	2.09 ± 0.21
$AUMC_{0\rightarrow\infty}$ (h•h•µg/mL)	70.25 ± 25.72	29.38 ± 3.42
Cl (mL/min/kg)	1.66 ± 2.21	NA
Vd_{ss} (L/kg)	0.65 ± 0.08	NA
λ_z (/h)	NA	0.14 ± 0.02
λ_z $t_{1/2}$ (h)	NA	4.94 ± 0.67
$t_{1/2\alpha}$ (h)	0.08 ± 0.05	NA
$t_{1/2\beta}$ (h)	4.56 ± 0.57	NA
K10 $t_{1/2}$ (h)	1.35 ± 0.97	NA
MRT (h)	NA	10.12 ± 1.54
C_{max} (µg/mL)	NA	0.14 ± 0.02
T_{max} (h)	NA	11.33 ± 1.63
T_{max} adjusted (h)	NA	10.95
K10 (/h)	2.78 ± 4.05	NA
K12 (/h)	8.57 ± 7.54	NA
K21 (/h)	2.15 ± 1.37	NA
A (µg/mL)	36.98 ± 54.51	NA
B (µg/mL)	1.60 ± 0.32	NA
α (/h)	13.35 ± 10.10	NA
β (/h)	0.15 ± 0.02	NA
Protein binding (%)	91.75 ± 0.63	NA
Partition coefficient	0.68 ± 0.05	NA

$AUC_{0\rightarrow\infty}$ = Area under the curve from time 0 to infinity. $AUMC_{0\rightarrow\infty}$ = Area under the first moment curve from time 0 to infinity. Cl = Clearance from the central compartment. Vd_{ss} = Apparent volume of distribution at steady state. λ_z = First-order rate constant of terminal portion of the curve. λ_z $t_{1/2}$ = Half-life of the terminal portion of the curve. $t_{1/2\alpha}$ = Distribution half-life. $t_{1/2\beta}$ = Elimination half-life. K10 $t_{1/2}$ = Half-life of elimination phase. MRT = Mean residence time. C_{max} = Maximum concentration. T_{max} = Time until maximum concentration. T_{max} adjusted = Time until maximal concentration adjusted for a 23 minute lag time. K10 = Elimination rate from compartment 1. K12 = Rate of movement from compartment 1 to compartment 2. K21 = Rate of movement from compartment 2 to compartment 1. A = Intercept for distribution phase. B = Intercept for elimination phase. α = Rate constant associated with distribution phase. β = Rate constant associated with elimination phase. NA = Not applicable for the route of administration or model used for estimations.

Table 2—Mean (± SD) values for pharmacokinetic parameters of meropenem after constant rate IV infusion (0.38 mg/kg/h) in 6 dogs

Parameters	Plasma	Interstitial fluid
$AUC_{0\rightarrow\infty}$ (h•µg/mL)	NA	7.26 ± 1.50
$AUMC_{0\rightarrow\infty}$ (h•h•µg/mL)	NA	47.83 ± 8.94
AUC_{par} (h·µg/mL)	9.92 ± 5.12	NA
Cl (mL/min/kg)	5.65 ± 2.76	NA
Vd_{ss} (L/kg)	0.30 ± 0.15	NA
λ_z (/h)	0.96 ± 0.09	0.31 ± 0.04
λ_z $t_{1/2}$ (h)	0.73 ± 0.07	2.31 ± 0.36
MRT (h)	NA	2.62 ± 0.45
C_{max} (µg/mL)	NA	0.86 ± 0.18
T_{max} (h)	NA	3.00 ± 1.67
T_{max} (h) (adjusted)	NA	2.62
C_{ss} (µg/mL)	1.39 ± 0.64	NA
Protein binding (%)	11.87	NA
Partition coefficient	0.02 ± 0.01	NA

AUC_{par} = Partial area under the curve calculated during the steady state period. C_{ss} = Mean concentration at steady state. *See* Table 1 for remainder of key.

the graphs. After administration of the loading dose or CRI of either drug, no adverse reactions were observed.

The length of the tubing connecting the ultrafiltration probe to the evacuated glass tube was custom made to a length of 11 cm by the manufacturer and held 38 µL of fluid. After ISF was collected from each dog, the amount of fluid and the duration of collection period were recorded. The mean rate of collection was 1.63 ± 0.34 µL/min. A lag time of 23 minutes was therefore determined for the collection of ISF and used to synchronize plasma and ISF samples. The ISF concentrations were adjusted for the 23-minute lag time (Fig 1 and 2), and the T_{max} values are reported as adjusted and not adjusted (Tables 1 and 2). A significant ($P < 0.001$) difference was found between ISF and plasma total doxycycline concentrations. No significant ($P = 0.395$) difference was found between ISF and plasma unbound doxycycline concentrations. Concentrations of meropenem in ISF and plasma (total and unbound) were similar. Determination of protein binding and lipophilicity revealed that, compared with meropenem, doxycycline has higher plasma protein binding (11.87%[6] vs 91.75 ± 0.63%) and lipophilicity (partition coefficients, 0.02 ± 0.01 vs 0.68 ± 0.05).

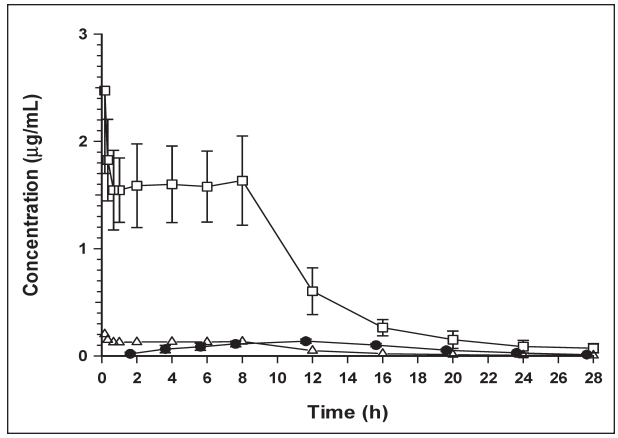

Figure 1—Plasma (total [open squares] and unbound [open triangles]) and interstitial fluid (ISF; solid circles) doxycycline concentrations versus time after constant rate IV infusion (CRI) of doxycycline in dogs. Plasma total and ISF doxycycline concentrations are expressed as mean (± SD) values. The ISF doxycycline concentrations have been corrected for the lag time (23 minutes) necessary for collection into an ultrafiltration device.

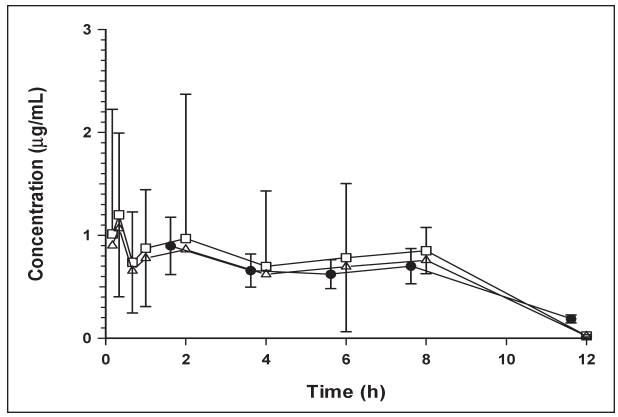

Figure 2—Plasma (total [open squares] and unbound [open triangles]) and ISF (solid circles) meropenem concentrations versus time after CRI of meropenem in dogs. Plasma total and ISF meropenem concentrations are expressed as mean (± SD) values. The ISF meropenem concentrations have been corrected for the lag time (23 minutes) necessary for collection into an ultrafiltration device.

Table 3—Physiochemical properties of doxycycline and meropenem

Properties	Doxycycline	Meropenem
Molecular weight	462.46	437.51
Protein binding (%)	91.75 ± 0.63	11.87
Partition coefficient	0.68 ± 0.05	0.02 ± 0.01
PKa[23,24]	3.09	7.40

PKa = Acid dissociation constant.

Discussion

The goal of our study was to evaluate the effect of high protein binding and lipophilicity (doxycycline) versus low protein binding and lipophilicity (meropenem) on the drug distribution between plasma and ISF at steady state. Meropenem pharmacokinetic parameters and protein binding have been estimated in a previously published study[6] by the authors.

The type of capillary bed influences the distribution of antimicrobials to the extravascular site.[20] Most capillary beds in the body are porous, allowing for free diffusion of molecules ≤ 1,000 d across the capillaries into the ISF of the extracellular space.[20,21] The CNS, retina, and prostate gland are examples of regions with nonporous capillary beds. The rate-limiting step for penetration to these nonporous tissues is lipophilicity, because diffusion is transcellular.[20,21] But for other tissues, protein binding limits passage across the membrane. Because the unbound fraction is responsible for microbiological activity and clinical efficacy, therapeutic failure of infections in the ISF treated with highly bound drugs (> 90% binding) can occur as a result of the unbound concentrations being less than the MIC for the organism.[22] Merrikin et al[4] evaluated the efficacy of several β-lactam antimicrobials against *Staphylococcus aureus* that were similar in MIC values and pharmacokinetic parameters but differed in the degree of protein binding. A nonlinear relationship existed between the extent of protein binding and antimicrobial activity. When the concentration of unbound drug decreased to < 10 to 20%, the efficacy decreased substantially.[4]

In our study, doxycycline and meropenem were chosen to compare the plasma pharmacokinetics and tissue distribution at steady state, because they differ in physiochemical properties that have been cited as important for drug distribution (**Table 3**).[23,24] Plasma protein binding was 91.75 ± 0.63% for doxycycline and 11.87% for meropenem.[6] Lipophilicity for doxycycline was higher (partition coefficient, 0.68 ± 0.05) than for meropenem (partition coefficient, 0.02 ± 0.01). Therefore, an aim of our study was to determine which of these factors, lipophilicity or protein binding, has a greater effect on tissue distribution.

Drug distribution is a dynamic process between plasma and the ISF. In contrast to a single bolus administration of a drug,[6] CRI allows an evaluation and comparison of the drug distribution to plasma and ISF under conditions in which the concentration gradient is kept constant. When plasma total and ISF doxycycline concentrations were compared during the steady state period, a significant ($P < 0.001$) difference was found between the concentrations. In contrast, when the plasma unbound doxycycline concentration was compared with the ISF doxycycline concentration, no significant ($P = 0.395$) difference was found. Clinically, it is the unbound fraction of the drug that is microbiologically active and able to diffuse through capillary pores to the target site, the ISF. Therefore, our results indicate that determining plasma doxycycline concentrations in dogs may not reflect the microbiologically active concentration of doxycycline in tissues at steady state.

Meropenem has low protein binding (11.87%).[6] Little difference is found between the plasma total and unbound meropenem concentrations and ISF meropenem concentrations. Therefore, the use of meropenem plasma pharmacokinetics in dogs may provide a good predictor of the microbiologically active concentration of meropenem in tissues at steady state.

The difference between plasma total and ISF concentrations for both drugs at steady state could be explained by differences in protein binding. Differences in lipophilicity appeared to have little importance for distribution to the ISF, because doxycycline had the higher lipophilicity but lower distribution to the ISF. Differences in elimination half-life were ruled out, because distribution was measured when each drug was at steady state.

Plasma Cl and Vd_{ss} estimated for meropenem in our study (5.65 ± 2.76 mL/min/kg and 0.30 ± 0.15 L/kg, respectively) were similar to previous reported values (6.53 ± 1.51 mL/min/kg and 0.34 ± 0.05 L/kg, respectively).[14] Plasma $t_{1/2}$ of meropenem (0.73 ± 0.07 hours) was also similar to a previous study[6] (0.69 ± 0.08 hours), but the ISF $t_{1/2}$ was longer in our study (2.31 ± 0.36 vs 1.15 ± 0.57 hours). In our study and the previous study,[6] the $t_{1/2}$ of meropenem is longer from ISF than from plasma, owing to the rapid plasma $t_{1/2}$. For a drug in the interstitial space to be eliminated, it must diffuse back into the plasma. If the time to equilibrium between tissue and plasma is longer than the time for the drug to be eliminated from plasma, a longer ISF $t_{1/2}$ of the drug will result.

The doxycycline plasma elimination $t_{1/2}$ (4.56 ± 0.57 hours) was similar to the ISF elimination $t_{1/2}$ (4.94 ± 0.67 hours), suggesting that time for equilibration between tissue and plasma is shorter or equal to plasma elimination. The plasma $t_{1/2\beta}$, Cl, and Vd_{ss} for doxycycline was 4.56 ± 0.57 hours, 1.66 ± 2.21 mL/min/kg, and 0.65 ± 0.08 L/kg, respectively, and was less than values reported in a study by Riond et al[10] (6.99 ± 1.09 hours, 1.72 ± 0.17 mL/min/kg, and 0.93 ± 0.14 L/kg, respectively). The Cl is higher for meropenem (5.65 ± 2.76 mL/min/kg) than doxycycline (1.66 ± 2.21 mL/min/kg) as a result of glomerular filtration and tubular secretion contributing to the elimination of meropenem.[25,26] In humans, a strong relationship is found between protein binding, renal clearance, and $t_{1/2}$ among tetracyclines.[27] The highly protein-bound doxycycline has a longer $t_{1/2}$ and lower renal clearance than tetracyclines with less extensive protein binding.[27] For β-lactams, no clear relationship is found between protein binding and $t_{1/2}$, because elimination involves tubular secretion, which is independent of protein binding.[22]

Lipophilicity is an important physiochemical property for determining intracellular concentrations of tetracyclines.[28] Because of the high lipophilicity of doxycycline, the unbound drug in the ISF and plasma is available to cross cell membranes and attain high intracellular concentrations. High intracellular concentrations account for the higher Vd_{ss} (0.65 ± 0.08 L/kg), compared with that of meropenem (0.30 ± 0.15 L/kg). As the lipid solubility of tetracyclines increases, the ratio of total drug in tissues to unbound drug in serum increases.[28]

Doxycycline binds reversibly to plasma proteins in humans, and these proteins are of low capacity; therefore, distribution depends upon the affinity of doxycycline for tissue components.[13] Protein binding remains a controversial topic in regards to therapeutic influence. However, as reviewed by Wise,[22] protein binding has an important effect on distribution, activity, and efficacy when it is high. Protein binding of different drugs can vary among species[29]; therefore, extrapolation of plasma and tissue protein binding, as well as volume of distribution and clearance, may be difficult among species.[30] The extent of protein binding depends on the drug, number of protein binding sites, compartment type, disease status, interaction with other agents, ionization, and lipophilicity of the drug.[3] Many of these factors are difficult to quantitate clinically, but they should be kept in mind when evaluating therapeutic efficacy.

The fluid collected from clinically normal dogs by the ultrafiltration device in our study may differ from the fluid surrounding an infected or inflamed tissue. Inflammation and infection may influence drug diffusion from capillaries by increasing the accumulation of protein, extracellular fluid, and cells. Bacteria present in the interstitial space can change the pH, protein content, and integrity of cellular barriers and, therefore, affect the interstitial distribution and intracellular concentrations.[31] Vasodilation and increased permeability accompanying inflammation causes an increase in drug delivery to the target site, but viscosity is also increased, slowing the rate of diffusion.[32] Drug elimination from infected sites may be prolonged as a result of protein binding at the site of inflammation from the presence of exudates.[32]

Tissue cages have been used to create a site with inflammation and infection and to study the dynamics of drug distribution.[32-35] But compared to the sample collection method used in our study, the tissue cage model represents a compartment with a small surface area-to-volume ratio as a result of the large sample collection space.[32,35] The in vivo ultrafiltration device obtains samples from the interstitial space, which has a large surface area-to-volume ratio and rapid equilibrium. Because of the small surface area-to-volume ratio in tissue cages, an artificially increased time-to-peak concentration and longer $t_{1/2}$ are seen with these devices.[35,36] In addition, drug concentrations measured in tissue cages are the total (bound and unbound) and may overestimate the microbiologically important fraction. However, we acknowledge that chronic tissue infection may change the usual extracellular environment as a result of fibrin barriers, abscess, edema, or changes in blood flow.[37] These factors are difficult to measure and quantify; therefore, it is not known how well the ultrafiltration ISF sample collection technique actually represents the distribution of antimicrobials into infected tissues.

Doxycycline and meropenem are 2 drugs with different physiochemical, pharmacokinetic, and protein binding characteristics. In our study, the difference between plasma total concentrations and ISF concentrations of both drugs could be explained by the extent of protein binding, rather than other factors such as lipophilicity, plasma $t_{1/2}$, and acid dissociation constant. When evaluating drug plasma pharmacokinetics for antimicrobials, protein binding should be taken into account when predicting efficacy. Disease processes that change drug plasma protein binding should be considered for antimicrobials when adjusting dosage regimens for drugs with high protein binding.

[a]Doxycycline, American Pharmaceutical Partners, Los Angeles, Calif.
[b]Merrem, Astra Zeneca, Wilmington, Del.
[c]Infusion pump (Baxter 6201), Universal Hospital Services, Moorisville, NC.
[d]Canine ultrafiltration probe (RUF-3-12), Bioanalytical Systems Inc, West Lafayette, Ind
[e]Centrifree, Millipore, Bedford, Mass.
[f]Doxycycline hydrochloride, Sigma Chemical Co, St Louis, Mo.
[g]Sodium phosphate dibasic, Sigma Chemical Co, St Louis, Mo.
[h]Phosphoric acid, Sigma Chemical Co, St Louis, Mo.
[i]l-octanol, Sigma Chemical Co, St Louis, Mo.
[j]Waters models 590 and 591, Waters, Franklin, Mass.
[k]Waters automated gradient controller, Waters, Franklin, Mass.
[l]Agilent Series 1050, Agilent Technologies, Wilmington, Del.
[m]Zorbax SB-C18, Agilent Technologies, Wilmington, Del.
[n]Spec Plus C18AR column filters, Ansys Diagnostics, Lake Forest, Calif.
[o]Ultrafree-MC, Millipore, Bedford, Mass.
[p]Zorbax SB-C8, Agilent Technologies, Wilmington, Del.
[q]Meropenem, Zeneca Pharmaceuticals, Wilmington, Del.
[r]WinNonlin, version 3.1, Pharsight, Cary, NC.
[s]SAS, version 8.2, SAS Institute Inc, Cary, NC.

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