CEFPODOXIME PROXETIL AND CEFALEXIN ARE THE MOST FREQUENTLY ADMINISTERED ORAL FORMULATIONS OF CEPHALOSPORINS IN DOGS. CEFPODOXIME IS A THIRD-GENERATION ORAL FORMULATION APPROVED FOR USE IN DOGS. CEFALEXIN IS A FIRST-GENERATION ORAL FORMULATION THAT IS USED IN AN EXTRALABEL MANNER IN DOGS. THE HUMAN GENERIC FORMULATION OF CEFALEXIN IS THE PRODUCT MOST OFTEN PRESCRIBED FOR USE IN DOGS. BOTH ANTIMICROBIALS ARE EFFECTIVE FOR THE TREATMENT OF BACTERIAL INFECTION IN DOGS. STUDIES HAVE REVEALED THE EFFICACY OF CEFALEXIN WHEN ADMINISTERED ORALLY FOR THE TREATMENT OF SKIN INFECTIONS (E.G., PYODERMA) IN DOGS AT DOSAGES BETWEEN 22 AND 35 MG/KG.

Pharmacokinetics, protein binding, and tissue distribution of orally administered cefpodoxime proxetil and cefalexin in dogs

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Objective—To determine the effect of protein binding on the pharmacokinetics and distribution from plasma to interstitial fluid (ISF) of cefalexin and cefpodoxime proxetil in dogs.

Animals—6 healthy dogs.

Procedures—In a crossover study design, 25 mg of cefalexin/kg or 9.6 mg of cefpodoxime/kg was administered orally. Blood samples were collected before (time 0) and 0.33, 0.66, 1, 2, 3, 4, 6, 8, 10, 12, 16, and 24 hours after treatment. An ultrafiltration device was used in vivo to collect ISF at 0, 2, 4, 6, 8, 10, 12, 16, and 24 hours. Plasma and ISF concentrations were analyzed with high-pressure liquid chromatography. Plasma protein binding was measured by use of a microcentrifugation technique.

Results—Mean plasma protein binding for cefpodoxime and cefalexin was 82.6% and 20.8%, respectively. Mean ± SD values for cefalexin in plasma were determined for peak plasma concentration (C max, 31.5 ± 11.5 µg/mL), area under the time-concentration curve (AUC, 155.6 ± 29.5 µg·h/mL), and terminal half-life (T ½, 4.7 ± 1.2 hours); corresponding values in ISF were 16.3 ± 5.8 µg/mL, 87.8 ± 21.0 µg·h/mL, and 3.2 ± 0.6 hours, respectively. Mean ± SD values for cefpodoxime in plasma were 33.0 ± 6.9 µg/mL (C max), 282.8 ± 44.0 µg·h/mL (AUC), and 5.7 ± 0.9 hours (T ½); corresponding values in ISF were 4.3 ± 2.0 µg/mL, 57.5 ± 17.4 µg·h/mL, and 10.4 ± 3.3 hours, respectively.

Conclusions and Clinical Relevance—Tissue concentration of protein-unbound cefpodoxime was similar to that of the protein-unbound plasma concentration. Cefpodoxime remained in tissues longer than did cefalexin. (Am J Vet Res 2010;71:1484–1491)
or cephalexin (26 mg/kg, PO, q 12 h) and treatment success was 96.8% and 93.9% for cefpodoxime-treated and cephalexin-treated dogs, respectively.

Pharmacokinetic studies in dogs have been reported for the oral administration of cefpodoxime and cephalexin, but these studies were limited to the measurement of total (protein-bound and protein-unbound fractions) plasma concentrations. In addition, investigators in these studies reported only the pharmacokinetics of plasma concentrations and did not indicate the concentration of drug at the biophase (site of infection) or determine the effect of protein binding on drug concentrations.

Antimicrobial experts have concluded that measurement of active drug concentrations in extracellular fluid is the preferred method to correlate pharmacokinetic-pharmacodynamic indices with clinical efficacy. In another study, investigators provided evidence that indicated antimicrobial concentrations at the target site (ie, ISF) are responsible for the antimicrobial effect and are more relevant than are plasma concentrations for predicting therapeutic efficacy. This conclusion was reiterated by other veterinary pharmacologists, who stated that plasma protein binding should be taken into account when examining pharmacokinetic factors because antimicrobial activity is dependent on the unbound drug concentration, rather than the total drug concentration, and that the protein-unbound drug concentration at the site of action (ie, ISF) is the most important predictor of therapeutic efficacy. Failure to recognize the differences between total plasma antimicrobial concentrations and active ISF antimicrobial concentrations may result in misinterpretation of pharmacokinetic-pharmacodynamic properties of a drug and its therapeutic efficacy.

Studies in dogs have revealed that drug concentrations in the ISF of tissues are determined more by plasma protein binding than by other chemical features of the drug. Investigators in those studies used an in vivo ultrafiltration device, which was a reliable and convenient method for collecting ISF samples from tissues in dogs. The use of an ultrafiltration device is the preferred method, rather than collection of tissue biopsy specimens or use of tissue cages, in animals because of ease of insertion and the ability to collect serial samples with the same device, without residual wounds or lesions after removal of the ultrafiltration probes. In addition, an ultrafiltration device provides a convenient method for continuous collection of samples and monitoring of drug distribution in unrestrained animals.

Other methods for measuring tissue drug concentrations in animals have traditionally included the processing of homogenized tissues or samples collected from implanted tissue cages. Pharmacology experts agree that the method of obtaining whole tissues, homogenizing them, and measuring the whole tissue concentration is of little value for understanding the relationship between tissue concentration and drug effect, particularly when evaluating this relationship in antimicrobials. The flaw in relying on this type of tissue concentration data was indicated in a review report. Evaluation of tissue biopsy specimens can overestimate lipophilic and underestimate hydrophilic drug concentrations because of the artificial mixing of intracellular fluid, blood, and tissue components during homogenization. In addition, because tissue biopsy specimens require that animals be euthanatized or that animals be anesthetized for biopsy procedures, it is difficult to achieve serial collection of samples for measurement of plasma-tissue fluid dynamics.

Tissue cages, which have been used by some investigators to estimate tissue drug concentrations, do not represent natural compartments. Furthermore, sedation, anesthesia, or both is required for cage implantation, and a 4- to 6-week waiting period is necessary before samples can be collected. Influx of fibrin into cages prevents repeated collection of samples and long-term use of the cages. The artificial compartments that arise from the use of tissue cages have a high volume because of the sample space within the device, and the actual volume of extracellular fluid of the tissues cannot be estimated from these compartments. Tissue cages are used to determine drug concentrations in fluids collected from tissues, but they typically misrepresent the true ISF concentrations because of delayed peaks and overestimated rates of drug elimination from tissues. In addition, drug concentrations in fluids collected in tissue cages may overrepresent the microbiologically active drug fraction in tissues because fluids include protein-bound and protein-unbound drug fractions, and only the protein-unbound fraction is microbiologically active.

The purpose of the study reported here was to determine the effect of protein binding on the pharmacokinetics and distribution of cephalexin and cefpodoxime from tissues to ISF in dogs and to compare observed drug concentrations with the MIC of Staphylococcus pseudintermedius (formerly Staphylococcus intermedius) and Escherichia coli.

Materials and Methods

Animals—Six adult mixed-breed dogs weighing between 19.8 and 32.3 kg (mean, 25.2 kg) were used in this study. Results of physical examination were used to determine that the dogs were healthy. Dogs were housed at the North Carolina State University Laboratory Animal Resources facility and fed a maintenance diet. The study was reviewed and approved by the Institutional Animal Care and Use Committee at North Carolina State University.

Study design—A 2-period, 2-treatment crossover design with a 7-day washout period between each treatment was used in this study. Dogs were assigned to the order in which they would receive treatments by random number selection. Cephalexin or cefpodoxime proxetil was administered orally. A mean dose of the base antimicrobial was calculated, which resulted in a dosage of 23 mg of cephalexin/kg or 9.6 mg of cefpodoxime proxetil/kg, respectively, administered orally as a single dose. Food was withheld for 18 hours before drug administration. Each dose was administered orally to each dog and immediately followed by administration of 12 mL of water to ensure the entire dose was swallowed.

Blood collection—Eighteen hours prior to drug administration, dogs were lightly sedated by the ad-
ministration of medetomidine hydrochloride (0.02 mg/kg, IV) and a catheter was inserted into a jugular vein of each dog. Catheters were flushed with sterile saline (0.9% NaCl) solution to maintain catheter patency. Blood samples were collected before (time 0) and 0.33, 0.66, 1, 2, 3, 4, 6, 8, 10, 12, 16, and 24 hours after administration of cephalixin and transferred into glass tubes containing lithium heparin (anticoagulant); additional blood samples were collected at 32 and 48 hours after administration of cefpodoxime because of an anticipated longer T1/2. Blood samples were immediately placed in ice and were later centrifuged at 1,000 g for 10 minutes. Plasma from centrifuged samples was separated and stored at –70°C.

ISF collection—Eighteen hours prior to the start of the study, an ultrafiltration probe was inserted into each dog. Interstitial fluid subsequently was collected by use of an in vivo ultrafiltration sampling kit. The ultrafiltration probe contained 3 loops with a 12-cm semipermeable membrane. The semipermeable membrane in the loop consisted of pores that allowed water, electrolytes, and low–molecular-weight molecules (<30 KDa) to diffuse across the membrane but excluded the passage of proteins and other high–molecular-weight compounds. The length of the nonpermeable tubing from the end of the 12-cm semipermeable membrane to the end of the external collection tube of the probe was 46 cm, and this 46-cm portion of the tube had a fluid capacity of 160 µL.

The ultrafiltration probe was inserted 18 hours prior to the start of the study to allow equilibration between the fluid in the ISF and the fluid collected into the ultrafiltration probe. Each probe (1 probe/dog) was inserted into the interstitial space overlying the lateral thorax. Although the dogs were still sedated after the administration of medetomidine, skin surrounding the insertion site of the probe was aseptically prepared and the insertion site was infused with a solution of 2% lidocaine hydrochloride. A guide needle was used to insert the ultrafiltration probe into the interstitial space under the skin so that the 3 loops of the probe remained under the skin in the interstitial space while the nonpermeable portion of the probe extended external to the dog’s skin. Once the ultrafiltration probe was in place, the guide needle was removed. An evacuated glass tube was connected to the nonpermeable portion of the probe to apply negative pressure on the probe system for ISF collection through the semipermeable membrane. After insertion of the ultrafiltration probe, sedation was reversed by the administration of atipamezole hydrochloride (0.3 mg/kg, IV). Dogs were fully recovered from sedation by the time of drug administration the following day. A new ultrafiltration probe was inserted (as described) in another location prior to each treatment period. Interstitial fluids were collected in conjunction with blood samples at the times described and immediately stored at –70°C. The mean ± SD volume of fluid collected into the evacuated glass tubes and the mean ± SD collection rate were calculated for each treatment period.

HPLC drug analysis—Plasma and ISF samples were analyzed via HPLC to determine the concentrations of cefpodoxime and cephalixin. The HPLC system consisted of a quaternary solvent delivery system (flow rate, 1 mL/min), an autosampler, and UV detector. Wavelengths of 235 and 265 nm were set for the UV detector for cefpodoxime and cephalixin, respectively. Chromatograms were integrated with a computer program. The analytic column was a reverse-phase, 4.6 X 15-cm C18 column that was maintained at a constant temperature (40°C). The mobile phase consisted of 85% distilled water and 15% acetonitrile. A 0.1% solution of trifluoroacetic acid was added to the mobile phase to lower the pH and improve the shape of the eluting peaks.

Cephalixin was measured as its base in plasma and ISF. Although cefpodoxime was administered as an ester, the parent drug is released after enzyme hydrolysis and cefpodoxime was measured in plasma and ISF. Reference standards of cephalixin hydrate and cefpodoxime were purchased or supplied, respectively, by the drug manufacturer. A stock solution of cefpodoxime (1 mg/mL) was prepared by dissolving the pure cefpodoxime reference standard in a 3:1 solution of 0.1% sodium bicarbonate to distilled water. A stock solution of cephalixin (1 mg/mL) was prepared by dissolving cephalixin hydrate in distilled water. Stock solutions were sealed and stored in the dark in a refrigerator.

Stock solutions were further diluted to create spiking solutions that would be used to fortify blank canine plasma and ISF for development of the HPLC method, calibration curves, and quality-control standards. Calibration curves for each drug consisted of 9 standard solutions that ranged between 0.05 and 30 µg/mL and included a blank (0 µg/mL) sample. The blank sample was used to detect interfering peaks that elute into the window of the chromatographic peak of interest and to measure background interference. The calibration curve was accepted if the linear coefficient of determination (r²) was ≥0.99 and if the calibration curve concentrations could be back-calculated to ≤15% of the true concentration of the standard.

All plasma, calibration, quality-control, and blank plasma samples were prepared in an identical manner. Solid-phase extraction cartridges were conditioned with 1 mL of methanol, which was followed by 1 mL of distilled water for cefpodoxime analysis or 1 mL of a 0.01M sodium acetate buffer for cephalixin analysis. Five hundred microliters of each plasma sample was added to a conditioned cartridge, which was followed by washing with 1 mL of a water-to-methanol (95:5) solution. The eluate from the cartridge was discarded. Then, drug was collected into a clean glass tube by elution with 1 mL of 100% methanol. The eluted samples were evaporated to yield a dry residue by heating the tubes at 40°C under a flow of air for 20 minutes. The residue of each tube was reconstituted by the addition of 200 µL of the mobile phase; solutions were vortexed briefly and transferred to an HPLC injection vial. Twenty-five microliters of each sample was used for injection into the HPLC system.

Retention time for cefpodoxime and cephalixin was 6.2 to 6.5 minutes and 7.2 to 7.4 minutes, respectively. Fresh calibration and blank samples were prepared for analysis each day. Limit of quantification for each drug
in canine plasma was 0.05 µg/mL, which was determined from the lowest point on a linear calibration curve that yielded an acceptable accuracy. All plasma samples had concentrations above the limit of quantification. Laboratory procedures were conducted in accordance with published guidelines on validation procedures.32

Because ISF samples were free of protein and other matrix components found in plasma, these could be injected directly into the HPLC system without prior preparation or extraction. For calibration of the standard curve, blank ISF was spiked with standard solutions of each drug to construct a calibration curve of 6 standard solutions that ranged between 0.10 and 20 µg/mL and included a blank (0 µg/mL) sample. All other conditions and criteria were identical to those described for the HPLC method for plasma sample analysis.

**Plasma protein binding**—Plasma protein binding was determined by use of an in vitro microcentrifugation system.1 Stock solutions of cephalexin and cefpodoxime were prepared by dissolving the drugs as described previously. Aliquots (3 mL) of pooled canine plasma were spiked with the stock solutions to generate cefpodoxime concentrations of 1, 5, 10, 15, 20, and 30 µg/mL. In addition, aliquots (3 mL) of pooled canine plasma were spiked with cephalexin to generate concentrations of 1 and 10 µg/mL. The samples were incubated and divided into 3 replicates, and 1 mL of each aliquot was added to a microcentrifugation system to obtain a protein-free ultrafiltrate in the reservoir for analysis by HPLC. Protein binding was determined by use of the following equation:

\[
\text{Protein binding} (\%) = \left( \frac{\text{[total concentration]} - \text{[protein-unbound concentration]}}{\text{[total concentration]}} \right) \times 100
\]

where total concentration is the sum of the protein-bound and protein-unbound drug concentration in plasma, and protein-unbound concentration is the protein-unbound drug concentration in plasma.

**MIC<sub>x</sub> comparison**—The MIC<sub>x</sub> values for *S. pseudintermedius* and *E. coli* were recorded for isolates collected from dogs in the United States and Europe for cephalexin31 and studies conducted in dogs and reported on the product label for cefpodoxime.6–11,e,f These MIC<sub>x</sub> values were used for comparison with observed plasma, ISF, and protein-unbound concentrations of cephalexin and cefpodoxime.

**Pharmacokinetic analysis**—Plasma and ISF drug concentrations were plotted on linear and semilogarithmic graphs for analysis and visual assessment of the best model for pharmacokinetic analysis. Analysis of curves and pharmacokinetic modeling were then performed by use of a commercial pharmacokinetic program.7 For noncompartmental analysis, AUC from time 0 to the last measured concentration was calculated by use of the log-linear trapezoidal method. The AUC from time 0 to infinity was calculated by adding the terminal portion of the curve, estimated from the relationship between \(C_n/\lambda\) to the AUC from time 0 to the last measured concentration, where \(\lambda\) is the terminal slope of the curve and \(C_n\) is the last measured concentration. Values for \(C_{\text{max}}\) and \(T_{\text{max}}\) after drug administration were determined directly from the data. The \(T_{1/2}\) was calculated from the terminal rate constant by use of the following equation:

\[
T_{1/2} = \frac{\ln 2}{\text{terminal rate constant}}
\]

where \(\ln\) is the natural logarithm. Secondary parameters were calculated in accordance with methods described elsewhere.33 Because of the length of the tubing, a \(T_{\text{lag}}\) was calculated for the amount of time required for ISF to collect in the evacuated glass tube, which was based on the length of the nonpermeable portion of the ultrafiltration probe and rate of collection. In addition, \(T_{\text{lag}}\) values were used to adjust the time for the reported antimicrobial concentration and \(T_{\text{max}}\) in the ISF for both antimicrobials by subtracting the \(T_{\text{lag}}\) from the time of sample collection. Plasma values that were dose dependent were calculated as the parameter per F. A tissue penetration factor was calculated by use of the following equation:

\[
\left( \frac{\text{AUC}_{\text{protein-unbound drug in ISF}}}{\text{AUC}_{\text{oral drug in plasma}}} \right) \times 100
\]

Relative bioavailability of cefpodoxime and cephalexin was calculated by use of the following equation:

\[
\text{Relative bioavailability} = \left( \frac{\text{AUC}_{\text{drug A}}}{\text{AUC}_{\text{drug B}}} \right) \times \left( \frac{\text{dose}_{\text{drug A}}}{\text{dose}_{\text{drug B}}} \right)
\]

**Statistical analysis**—We conducted a review of the literature and identified pharmacokinetic studies of cefpodoxime proxetil5 and cephalexin6–11,e,f in dogs. Data were pooled from pharmacokinetic studies6–11,e,f of cephalexin. Least squares means were calculated to account for differences in the number of observations for each study of cephalexin and reported as weighted mean values. This calculation generated overall mean values among pharmacokinetic studies while considering both the intrastudy and interstudy variation. These weighted mean values then were used for comparison with pharmacokinetic results of the present study. Values for CI/F and the apparent volume of distribution per absolute fraction of the dose absorbed after oral administration (ie, Vd/F) were not included in the study8 of cefpodoxime but were estimated from the pharmacokinetic data and by use of equations described elsewhere.33

**Results**

Mean \(T_{\text{lag}}\) for ISF collection after cefpodoxime and cephalexin administration was 3.3 and 2.15 hours, respectively. Adjustments were made to the ISF drug concentration time points by use of the respective calculated \(T_{\text{lag}}\) and semilogarithmic concentration-time curves were constructed for plasma, ISF, and protein-unbound concentrations of both antimicrobials (Figures 1 and 2). Compartmental analysis was attempted. However, after it became obvious that there was no consistent compartmental model that would be suitable for all the animals in the study, noncompartmental analysis was used because this type of analysis does not assume any compartmental structure. Pharmacokinetic parameters determined by use of noncompartmental analysis for
Plasma protein binding of cefpodoxime ranged between 9% and 34%. Mean overall protein-unbound fraction across the entire range of cefpodoxime concentrations was 17%. Mean ± SD tissue penetration factor for cefpodoxime was 20.5 ± 5.6%. A close agreement between protein-unbound drug concentrations in plasma and protein-unbound cefpodoxime concentrations in ISF was observed; the largest discrepancy was at the highest concentrations, where there appeared to be concentration-dependent protein binding (Figure 1; Table 3).

Plasma protein binding of cephalixin ranged between 25.9% and 15.7% at 1 and 10 µg/mL, respectively (Table 3). Furthermore, this represented a protein-unbound fraction of cephalixin ranging between 74.1% and 84.3% or an overall protein-unbound fraction of 79%. Mean ± SD tissue penetration factor of cephalixin was 58.2 ± 17.0%, which was slightly less than the value predicted by protein binding. Relative bioavailability of cefpodoxime, compared with cephalixin, was 4.73 times as high as that of cephalixin. The relationship between observed plasma, ISF, and protein-unbound concentrations and the MIC₉₀ of S pseudintermedius and E coli after oral administration of a mean dose of 9.6 mg of cefpodoxime proxetil/kg was compared (Figures 1 and 2). Mean protein-unbound concentration of cephalixin in ISF exceeded the MIC₉₀ for S pseudintermedius (2 µg/mL) and E coli (16 µg/mL) for approximately 12 hours after drug administration. However, it did not achieve the MIC₉₀ for cefpodoxime (0.5 µg/mL).

### Table 1—Mean ± SD values for pharmacokinetic parameters of cefpodoxime after oral administration of a mean dose of 9.6 mg of cefpodoxime proxetil/kg in 6 adult dogs.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>ISF*</th>
<th>Total plasma†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Terminal rate constant (/h)</td>
<td>0.07 ± 0.02</td>
<td>0.12 ± 0.02</td>
</tr>
<tr>
<td>Tₚ (h)</td>
<td>10.38 ± 0.32</td>
<td>5.75 ± 0.91</td>
</tr>
<tr>
<td>Tₚ (h)</td>
<td>3.27 ± 0.06</td>
<td>3.10 ± 0.11</td>
</tr>
<tr>
<td>Cₚₚ (µg/mL)</td>
<td>4.33 ± 1.96</td>
<td>32.96 ± 6.92</td>
</tr>
<tr>
<td>AUC (h • µg/mL)</td>
<td>57.47 ± 17.45</td>
<td>282.84 ± 44.05</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>17.31 ± 2.42</td>
<td>—</td>
</tr>
<tr>
<td>Tissue penetration factor (%)</td>
<td>20 ± 0</td>
<td>—</td>
</tr>
<tr>
<td>VD/F (mL/kg)</td>
<td>—</td>
<td>288.59 ± 65.58</td>
</tr>
<tr>
<td>CL/F (mL/kg/h)</td>
<td>—</td>
<td>34.63 ± 4.92</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>—</td>
<td>5.77 ± 1.48</td>
</tr>
</tbody>
</table>

* A Tₚₚ was calculated for the collection of ISF because of the length of the nonpermeable portion of the tubing of the in vivo ultrafiltration probe. The Tₚₚ value then was used to adjust the reported concentrations of cefpodoxime in ISF. The MIC₉₀ (0.5 µg/mL; dashed line) for Staphylococcus pseudintermedius and Escherichia coli reported in another study³¹ is provided for comparison with the observed cefpodoxime concentrations.

### Table 2—Mean ± SD values for pharmacokinetic parameters of cephalixin after oral administration of a mean dose of 25 mg/kg in 6 adult dogs.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>ISF*</th>
<th>Total plasma†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Terminal rate constant (/h)</td>
<td>0.23 ± 0.05</td>
<td>0.15 ± 0.03</td>
</tr>
<tr>
<td>Tₚ (h)</td>
<td>3.19 ± 0.63</td>
<td>4.74 ± 1.15</td>
</tr>
<tr>
<td>Tₚ (h)</td>
<td>2.07 ± 0.56</td>
<td>2.83 ± 1.72</td>
</tr>
<tr>
<td>Cₚₚ (µg/mL)</td>
<td>16.34 ± 5.82</td>
<td>31.52 ± 11.49</td>
</tr>
<tr>
<td>AUC (h • µg/mL)</td>
<td>67.79 ± 21.02</td>
<td>155.63 ± 29.53</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>7.51 ± 1.06</td>
<td>—</td>
</tr>
<tr>
<td>Tissue penetration factor (%)</td>
<td>58 ± 17</td>
<td>—</td>
</tr>
<tr>
<td>VD/F (mL/kg)</td>
<td>—</td>
<td>1,162.12 ± 431.88</td>
</tr>
<tr>
<td>CL/F (mL/kg/h)</td>
<td>—</td>
<td>188.17 ± 29.99</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>—</td>
<td>5.50 ± 2.07</td>
</tr>
</tbody>
</table>

See Table 1 for key.
Pharmacokinetic results of the present study were compared with results of other studies\(^8\text{--}^{16}\) (Table 4). For cephalexin, mean \(T_{1/2}\) and \(C_{\text{max}}\) in the present study were longer and higher, respectively, than those in other studies, even though similar doses were administered. For cefpodoxime, a similar mean \(T_{1/2}\) was reported. However, a higher \(C_{\text{max}}\), by almost a 2-fold factor, was observed in the present study.

### Discussion

Although other pharmacokinetic studies have been reported on these antimicrobials in dogs, to our knowledge, the study reported here is the first in which ISF concentrations of protein-unbound cefpodoxime or cephalexin were measured in dogs. Because it is the concentration of the protein-unbound antimicrobial in the tissue biophase (site of bacterial infection) that is important for drug action, these data are relevant for predicting antimicrobial activity. In addition, the present study revealed that when plasma protein binding is taken into consideration, the measure of protein-unbound cefpodoxime in plasma is predictive of the protein-unbound drug concentration in the extracellular fluid (ISF) in tissues. Similar to another study\(^3\) with cephalosporins, we calculated a tissue penetration factor to represent the relationship between unbound drug concentrations in ISF and the total plasma drug concentration. This factor was calculated from the ratio of protein-unbound drug concentration in ISF to the total drug concentration in plasma. The protein-unbound drug concentration in plasma should be in equilibrium with the protein-unbound drug concentration in tissue, which was apparent for cefpodoxime in which these values were approximately 17\% and 20\%, respectively. This is also apparent from a visual examination of the concentrations, in which there is close similarity between ISF drug concentration and the tissue penetration factor, compared with the lower penetration factor, compared with the protein-unbound drug concentration in plasma, is that cefpodoxime is eliminated faster than is cephalexin after administration of a single dose. Because tissue and plasma concentrations were not measured at steady-state concentrations, equilibrium may not have been achieved. A drug that is eliminated more rapidly (shorter \(T_{1/2}\)) will not have as much time to achieve equilibrium between plasma and tissue, compared with a drug with slower elimination or compared with a drug that has achieved a steady-state concentration. This principle was described in a review\(^35\) of cephalosporins, in which a clear asso-

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Cephalexin</th>
<th>Cefpodoxime</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL/F (mL/kg/min)</td>
<td>2.8 ± 0.5</td>
<td>0.58 ± 0.08</td>
</tr>
<tr>
<td>VD/F (L/kg)</td>
<td>1.16 ± 0.43</td>
<td>0.09 ± 0.38</td>
</tr>
<tr>
<td>(T_{1/2}) (h)</td>
<td>4.74 ± 1.15</td>
<td>5.75 ± 0.91</td>
</tr>
<tr>
<td>(C_{\text{max}}) (µg/mL)</td>
<td>31.52 ± 11.49</td>
<td>32.96 ± 6.92</td>
</tr>
</tbody>
</table>

**Table 3.—Protein-bound and protein-unbound fractions of cefpodoxime and cephalexin determined in blank canine plasma samples spiked with known concentrations of cefpodoxime or cephalexin.**

<table>
<thead>
<tr>
<th>Concentration (µg/mL)</th>
<th>Cefpodoxime</th>
<th>Cephalexin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>83.06 ± 3.54</td>
<td>25.94 ± 5.14</td>
</tr>
<tr>
<td>5</td>
<td>91.25 ± 0.38</td>
<td>74.06</td>
</tr>
<tr>
<td>10</td>
<td>89.33 ± 2.44</td>
<td>15.74 ± 6.4</td>
</tr>
<tr>
<td>15</td>
<td>82.41 ± 0.97</td>
<td>84.26</td>
</tr>
<tr>
<td>20</td>
<td>83.60 ± 0.77</td>
<td>—</td>
</tr>
<tr>
<td>30</td>
<td>66.17 ± 0.30</td>
<td>—</td>
</tr>
<tr>
<td>Overall mean</td>
<td>82.64</td>
<td>20.84</td>
</tr>
</tbody>
</table>

**Table 4.—Comparison between values for the pharmacokinetic parameters reported in the present study and those reported in other studies for cefpodoxime proxetil and cephalexin when administered orally at similar doses in dogs.**

**Discussion**

Although other pharmacokinetic studies have been reported on these antimicrobials in dogs, to our knowledge, the study reported here is the first in which ISF concentrations of protein-unbound cefpodoxime or cephalexin were measured in dogs. Because it is the concentration of the protein-unbound antimicrobial in the tissue biophase (site of bacterial infection) that is important for drug action, these data are relevant for predicting antimicrobial activity. In addition, the present study revealed that when plasma protein binding is taken into consideration, the measure of protein-unbound cefpodoxime in plasma is predictive of the protein-unbound drug concentration in the extracellular fluid (ISF) in tissues. Similar to another study\(^3\) with cephalosporins, we calculated a tissue penetration factor to represent the relationship between unbound drug concentrations in ISF and the total plasma drug concentration. This factor was calculated from the ratio of protein-unbound drug concentration in ISF to the total drug concentration in plasma. The protein-unbound drug concentration in plasma should be in equilibrium with the protein-unbound drug concentration in tissue, which was apparent for cefpodoxime in which these values were approximately 17\% and 20\%, respectively. This is also apparent from a visual examination of the concentrations, in which there is close similarity between ISF drug concentration and the protein-unbound drug concentration in plasma after a single dose of cefpodoxime (Figure 1).

Mean tissue penetration factor of cephalexin was 58\%, but the mean protein-unbound drug concentration in plasma was 79\%. A plausible explanation for the lower penetration factor, compared with the protein-unbound drug concentration in plasma, is that cefpodoxime is eliminated faster than is cephalexin after administration of a single dose. Because tissue and plasma concentrations were not measured at steady-state concentrations, equilibrium may not have been achieved. A drug that is eliminated more rapidly (shorter \(T_{1/2}\)) will not have as much time to achieve equilibrium between plasma and tissue, compared with a drug with slower elimination or compared with a drug that has achieved a steady-state concentration. This principle was described in a review\(^35\) of cephalosporins, in which a clear asso-

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cation was detected between the percentage of tissue penetration and the drug’s T1/2.

As reported by investigators in other studies,16,18,23,34 tissue distribution is influenced more by protein binding than by other chemical features of the drug. These results agreed with the results of another study34 of the distribution of cefpodoxime in humans. In that study,34 microdialysis was used to collect protein-free drug in ISF, whereas this study in dogs used ultrafiltration. Both methods are based on the principle of collecting protein-unbound ISF by use of an implanted dialysis probe in the tissue. In the study34 in humans, tissue penetration factors for cefpodoxime and cefixime were related to plasma protein binding, similar to our observations in the study reported here. In addition, that study34 revealed that mean ± SD plasma protein binding and plasma T1/2 for cefpodoxime were 21 ± 4% and 2.6 ± 0.4 hours, respectively. In the study reported here, mean ± SD plasma T1/2 was 5.73 ± 0.91 hours and mean plasma protein binding was 82.64%. This agrees with observations made by investigators of another study35 in that the more highly protein-bound cephalexin are eliminated more slowly than are the cephalexin that are less protein bound.

Cephalosporins are time-dependent drugs for which antimicrobial efficacy is based on the duration of time that the drug concentrations of cefpodoxime and cephalexin exceed the MIC.36 A difference was observed in the duration that antimicrobial drug concentrations exceed the MIC90 of S pseudintermedius and E coli. The mean protein-unbound drug concentrations of cefpodoxime in the ISF exceeded the MIC90 of S pseudintermedius and E coli for approximately 24 hours (Figure 1). Conversely, mean cephalaxin concentration in ISF exceeded the MIC90 for S pseudintermedius for approximately 12 hours, but it did not achieve concentrations greater than the MIC90 of E coli (Figure 2). Cefpodoxime is approved for use in dogs with a dosing interval of 24 hours, whereas most clinicians in the United States administer cephalexin to dogs every 12 hours. There was equal efficacy for treatment of bacterial pyoderma in 137 dogs when cefpodoxime was administered orally once daily and cephalexin was administered orally twice daily.4 The study reported here revealed the advantages of ISF collection by use of an in vivo ultrafiltration technique. This technique allowed for serial collection of samples of ISF that could be evaluated for drug concentrations and compared with plasma drug concentration. This technique also avoided the problem associated with interpretation of results generated from the measurement of ISF concentrations via processing of homogenized tissues or samples collected from implanted tissue cages. In addition, use of this procedure avoided the need to anesthetize the dogs and collect surgical biopsies to determine drug tissue concentration.

Review of the literature identified pharmacokinetic studies of cefpodoxime5 and cephalexin6–11,14 in dogs that were used for comparison with the pharmacokinetics of the present study (Table 4). For cephalexin, mean T1/2 and Cmax were longer and higher, respectively, for the study reported here. The F of cephalexin was not reported in those studies6–11,14; therefore, the reasons for the discrepancies in T1/2 and Cmax are not known, despite the fact that similar doses were administered. For cefpodoxime, a similar T1/2 and a greater Cmax were observed in the present study, despite administration of a similar dose in the study4 used for comparison. Investigators of that study4 reported an approximate F of 33% after oral administration of cefpodoxime. The AUC of cefpodoxime in the present study was approximately 1.8 times as high. It is possible that F in the present study was much greater.

Three properties that favor the use of cefpodoxime when treating bacterial infections are high protein binding, high antimicrobial activity (low MIC because it is a third-generation cephalosporin), and time-dependent antimicrobial activity. High protein binding contributes to a longer T1/2, which when coupled with lower MIC values, yielded protein-unbound drug concentrations in tissues that were maintained longer than for cephalexin (Figures 1 and 2). Because cephalosporins are time-dependent drugs, the long duration of active drug concentrations allows for once-daily administration. The duration of protein-unbound drug concentrations in tissues of the study reported here appears to agree with that of a clinical study4 in which investigators evaluated the efficacy of both of these antimicrobials.
References