Research Article

Saturable Processes Affecting Renal Clearance of Cefixime in Dogs

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The pharmacokinetics of cefixime, a new orally active cephalosporin, was studied after an intravenous dose of 50 mg/kg to four beagle dogs. Cefixime was shown to exhibit concentration dependent serum protein binding and saturable tubular reabsorption. The drug was excreted mainly in the urine, the net result of glomerular filtration and saturable tubular reabsorption. The experimental results were analyzed by model independent pharmacokinetic equations and with theoretical models describing renal clearance. Modification of the models, based on observed data, was proposed. The experimental methods employed and the pharmacokinetic approach offered in this study can be applied to drugs that exhibit concentration dependent protein binding and saturable renal elimination processes.

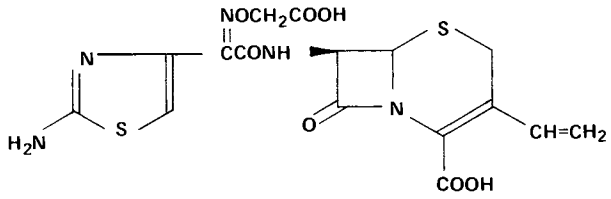
KEY WORDS: renal clearance; cephalosporin; cefixime; tubular reabsorption; saturable protein binding; pharmacokinetics.

INTRODUCTION

Cefixime {CL 284,635; FK 027; (6R, 7R)-7-[(Z)-2-(2-amino-4-thiazolyl) -2- (carboxymethoxyimino)acetamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo- (4.2.0) -oct-2-ene-2-carboxylic acid—Scheme 1} is a novel, third-generation, orally active cephalosporin undergoing advanced clinical trials for the treatment of urinary and respiratory tract infections (1–3). The drug is effective against a broad spectrum of gram-negative and gram-positive bacteria.

During the course of cefixime's development, extensive animal testing and pharmacokinetic support were provided in toxicology safety evaluation, formulation development, and clinical support studies. Pharmacokinetic analyses were necessary for optimal interpretation of the data and to identify and understand species differences in cefixime's disposition. Rats, rabbits, and dogs were the primary test species employed in these studies.

In dogs, cefixime presents an interesting pharmacokinetic profile. It is eliminated primarily by the kidney, its renal clearance a composite of filtration at the glomerulus and reabsorption at the proximal tubules (4,6). Cefixime exhibits a concentration-dependent protein binding in dog serum. It is highly protein bound (93%) at therapeutically relevant serum concentrations of less than 30 μg/ml. However, at higher serum concentrations routinely encountered during bioavailability and toxicology safety evaluation studies, serum free fractions (f_u), at 300 μg/ml (9,10), have exceeded 45%. The extensive protein binding and tubular reabsorption at cefixime at the lower serum concentrations result in a relatively long half-life of 7 hr, while at higher concentrations, saturation of the binding and reabsorption processes is thought to cause the observed nonlinear kinetics in dogs.



Scheme 1.

Previous dog studies had shown that cefixime exhibits dose-dependent pharmacokinetics in the areas of bioavailability (F), clearance, and volume of distribution (7). Fourfold increases in intravenous doses (12.5–50 mg/kg) resulted in a doubling of renal clearance and volume of distribution values. Discrepancies in F calculated by serum AUC data versus F calculated with urinary excretion values were also observed in these studies and suggested nonlinear elimination processes for cefixime. Following the iv administration of extremely high doses of cefixime to dogs—as part of a range-finding toxicology safety evaluation study—dose- and time-dependent changes in renal clearance and volume of distribution were observed along with disproportionate increases in AUC with increasing doses (11).

The objective of this study was to examine the relationship among protein binding, tubular reabsorption, and the apparent nonlinearities of cefixime kinetics in dogs. In so

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doing, methodology was developed that permitted these studies to be conducted as part of the preclinical development program for this new cephalosporin antibiotic.

MATERIALS AND METHODS

Following an overnight fast, four beagle dogs were administered 50 mg/kg of ^{14}C-cefixime as an intravenous bolus dose. The specific activity and radiopurity of the ^{14}C-labeled drug were 0.36 μCi/mg and >95%, respectively; the labeled carbon was at position 2 of the thiazole ring.

Blood samples (5 ml) were obtained from the jugular vein at 0, 5, 15, 30, 45, and 60 min and 1.5, 2.5, 3.5, 5, 7, 9, 11, 16, 24, 29, 36, and 48 hr after dosing. They were chilled for 1 hr (~4°C) to promote clot formation and centrifuged at 3000 rpm for 20 min. To avoid repeated freezing and thawing of samples, sera were separated into three aliquots; one was used for liquid scintillation counting, the second for protein binding determination, and the third for high-pressure liquid chromatographic (HPLC) analysis of cefixime. These samples were stored frozen at −20°C. Drug concentrations in serum and urine were measured by scintillation counting of radioactivity and by a specific and sensitive HPLC assay (12,13). The analytical results obtained by the two assay methods were equivalent over the 12 hr for which the dogs were catheterized. Serum and urine concentrations used for computational purposes were obtained from HPLC values.

To ensure quantitative urine recovery, essential for clearance calculations, all four dogs were pretrained to stand at ease in dog slings (Alice King Catham Medical Arts, Los Angeles) for 12 hr. One hour prior to dosing, the dogs were bladder-catheterized using Swan-Ganz flow-directed, double-lumen cardiac catheters (American Edward Labs, Amasco, Puerto Rico). During each collection period, urine was allowed to drain passively into graduate cylinders. At the end of each interval, the catheters and bladder were rinsed two or three times with 5 ml of water which was added to the urine collected directly. To replace water loss during blood sampling and to maintain urine flow, approximately 10 ml of water was administered orally to the dogs hourly from −1 to 12 hr postdose. Urine was collected in dog metabolism cages at all time periods after 12 hr.

The *in vivo* protein binding of cefixime was determined in all serum samples by equilibrium dialysis. One milliliter of serum was dialyzed against 1 ml of isotonic phosphate buffer, pH 7.4, in 1-ml chambers. Dialysis was across a Spectra Por 2 dialysis membrane with a molecular weight cutoff of 12,000–14,000 (Spectrum Medical Industries, McGaw Park, Ill.). The cells were rotated slowly (5 rpm) for 6 hr at 37°C. Previous studies had shown that these conditions were suitable to attain equilibrium (10).

Calculations of protein binding values was a two-step process. First, drug concentrations on the buffer and serum side at equilibrium (postdialysis) were measured by radioactive counting. The fraction unbound (f_u) postdialysis was defined as the quotient of drug concentration in the buffer divided by the drug concentration in the serum (postdialysis). Second, the effects of volume shifts and loss of drug from the serum chamber during the experiment—two natural consequences of the dialysis procedure (14,15)—were assessed. Volume shifts, buffer to serum, were low (~5%) and subsequently no corrections were made for them. However, significant transfer of free drug to the buffer chamber did occur. Consequently, the initial f_u measured binding at reduced postdialysis serum concentrations and was not directly representative of predialysis binding. Correction to predialysis values was made with binding curves generated following least-squares regression analysis of postdialysis serum concentrations versus their corresponding fu values. Free-fraction values, corresponding to predialysis serum concentrations, were obtained by interpolation from the fitted curves for the individual dogs.

This method is similar to that used by Giacomini *et al.* (16) with the following modification. Their binding curves were generated *in vitro* by adding increasing amounts of test compound to their subjects' serum, obtained prior to dosing. For cefixime the binding curves were derived directly from serum which contained drug as a result of dosing.

RESULTS AND DISCUSSION

Figure 1 depicts the mean concentrations of total and free cefixime in serum. The mean urinary excretion rate versus time is shown in Fig. 2. The mean urinary excretion rate ($\Delta X_u/\Delta t$), the renal clearance of the total (CL_r) and free (CL_{rf}) drug, and the free fraction (f_u) of the drug during the individual time intervals are listed in Table I. Of the administered dose, 71.6 ± 6.6% (mean ± SD) was eliminated in the urine during the first 12 hr. Of this, about 90% was excreted in the urine as unchanged drug. The mean CL_r over the 0- to 12-hr period decreased from an initial high of 1.64 ± 0.53 ml/min/kg to a low of 0.33 ± 0.1 ml/min/kg or from 13.1 to 2.7 ml/min (an 80% decrease). In contrast, the mean CL_{rf} was relatively constant (mean value, 3.31 ± 0.77 ml/min/kg or 28.2 ± 5.6 ml/min). The only significant change, a 47% decrease, was noticed when the first and last values of CL_{rf} were compared. Excluding the first point, the results suggest a more stable renal clearance of free drug over the 12 hr for which the dogs were catheterized. The decrease in CL_r was correlated with the decrease in the *in vivo* f_u from 33.6 ± 3.7 to 10.4 ± 3.1% (Table I). CL_r was calculated using Eq. (1) (17).

$$CL_r = \frac{\Delta X_u}{\Delta t} / C \quad (1)$$

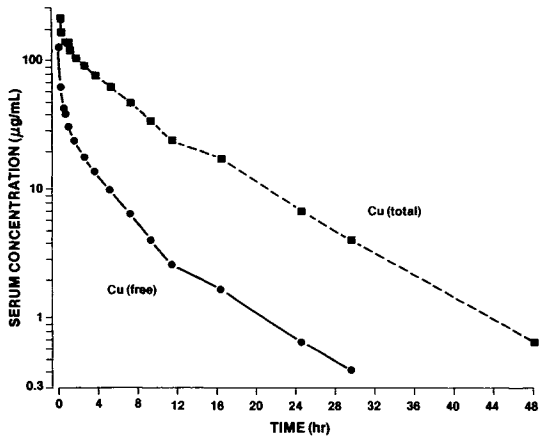

Fig. 1. Mean free and total serum cefixime concentrations following a 50-mg/kg intravenous dose in four dogs.

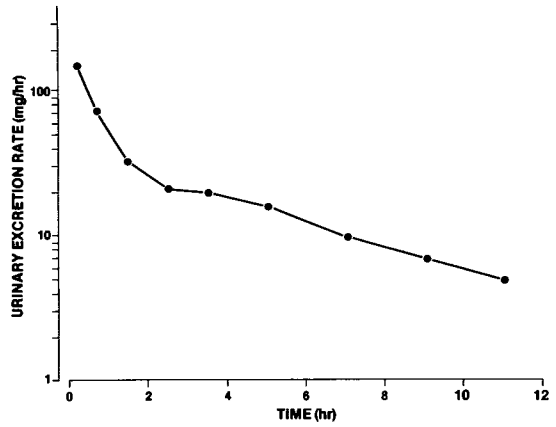

Fig. 2. Mean urinary excretion rate versus time for cefixime in dogs.

where ΔX_u is the amount excreted in the urine over the time interval Δt and C is the drug serum concentration at the midpoint of Δt.

When the ability of the kidney to eliminate the drug is smaller than the rate of drug delivery to the kidney (as it is in the case of cefixime), CL_r can be estimated by the product of the free fraction (f_u) and the renal clearance of the free (unbound) drug (CL_{rf}) [Eq. (2)] (17,18).

$$CL_r = f_u\, CL_{rf} = \frac{f_u \Delta X_u}{C_u \Delta t} \tag{2}$$

where C_u is the free (unbound) drug concentration in the serum and

$$\frac{\Delta X_u}{\Delta t} = CL_{rf}\, C_u \tag{3}$$

Figure 3 depicts the plot of $\Delta X_u/\Delta t$ versus C and C_u for the individual dogs; two distinct phases are present. At total concentrations below ~100 μg/ml, a linear relationship between urinary excretion rate and C was obtained. At total concentrations above ~100 μg/ml, the urinary excretion rate increased disproportionately with increases in C.

When the urinary excretion rate is directly related to C_u, and there is concentration-dependent protein binding, normalization to free drug concentrations should result in a straight line. In this investigation, a plot of $\Delta X_u/\Delta t$ versus C_u, also shown in Fig. 3, did not completely linearize this relationship. This suggests that, in addition to the concentration-dependent protein binding, a second saturable process is contributing to the renal clearance of cefixime. Previous reports, in which stop-flow methodology was employed to study the renal clearance of cefixime, indicated that in addition to glomerular filteration, cefixime undergoes extensive tubular reabsorption (~50%) but no tubular secretion (4). This process was not saturable at concentrations below 75 μg/ml.

Drugs which exhibit saturable tubular reabsorption are depicted by Garrett (19) as being concave with respect to the $\Delta X_u/\Delta t$ versus plasma concentration curves—a feature similar to that observed in this study. The relationship derived by Garrett for a drug undergoing renal excretion and saturable tubular reabsorption is given in Eq. (4).

$$\frac{\Delta X_u}{\Delta t} = GFR\, C - \frac{K_{tr}\, k_{tr}\, V\, C}{1 + K_{tr}\, V\, C} \tag{4}$$

where GFR is the glomerular filteration rate, K_{tr} is the degree of saturability in the reabsorption process, V is the volume of distribution, k_{tr} is the maximum tubular reabsorption rate, and C is the total drug concentration in serum or plasma.

This equation is useful for describing the relationship between the urinary excretion rate of drugs which are not extensively bound to plasma (serum) proteins and do not exhibit concentration-dependent protein binding. It is currently accepted that the urinary excretion rate of most drugs (especially drugs with low extraction ratio) is governed by the concentration of free drug in plasma rather than the total concentration. To account for the free concentration in serum Eq. (4) can be expressed, with C_u replacing C, as follows:

$$\frac{\Delta X_u}{\Delta t} = GFR\, C_u - \frac{K_{tr}\, k_{tr}\, V\, C_u}{1 + K_{tr}\, V\, C_u} \tag{5}$$

This modification does not necessarily mean that the term C, as suggested by Eq. (4), is replaced by C_u/f_u. At the

Table I. Urinary Recovery, Renal Clearance of Total (CL_r) and Free (CL_{rf}), and Free Fraction (f_u) of Cefixime During Selected Time Intervals (Mean ± SD; $N = 4$)

Time interval (hr)	Recovery (%)	CL_r (ml/min/kg)	CL_{rf} (ml/min/kg)	f_u (%)[a]
0–0.5	20.1 ± 3.4	1.64 ± 0.53	4.99 ± 1.84	33.6 ± 3.7
0.5–1	8.9 ± 1.1	0.95 ± 1.4	3.99 ± 0.94	24.2 ± 3.3
1–2	10.5 ± 2.3	0.63 ± 0.22	3.31 ± 0.81	20.1 ± 3.8
2–3	6.7 ± 1.2	0.46 ± 0.14	2.47 ± 0.68	18.1 ± 3.4
3–4	5.3 ± 0.3	0.44 ± 0.10	2.82 ± 0.70	16.1 ± 3.3
4–6	8.4 ± 0.2	0.40 ± 0.10	3.15 ± 0.28	14.5 ± 3.2
6–8	5.3 ± 0.3	0.38 ± 0.11	3.16 ± 0.96	12.4 ± 3.2
8–10	3.9 ± 0.5	0.36 ± 0.11	3.26 ± 0.88	11.2 ± 3.3
10–12	2.5 ± 0.5	0.33 ± 0.1	2.64 ± 0.32	10.4 ± 3.1

[a] Determined for plasma samples at midtime.

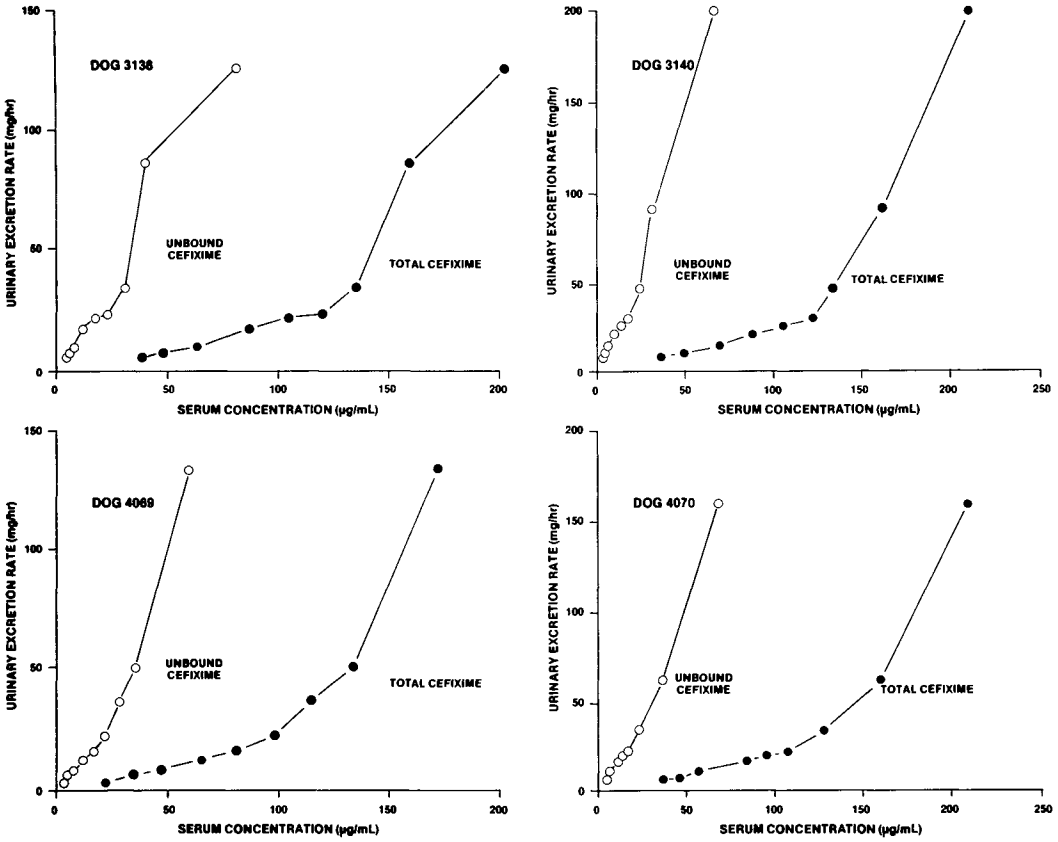

Fig. 3. Urinary excretion rate versus free and total serum concentrations of cefixime in individual dogs.

highest concentration when $K_{tr}\ V\ C_u >>> 1$, Eq. (5) can be simplified to Eq. (6).

$$\frac{\Delta X_u}{\Delta t} = C_u\ \text{GFR} - k_{tr} \tag{6}$$

Equations (5) and (6) assume that the concentration of the drug in the tubular urine is proportional to the free serum concentration and not the total concentration (because f_u changes with concentration). Equation (6), valid at higher serum concentrations, shows that a plot of $\Delta X_u/\Delta t$ versus C_u will yield a straight line which no longer passes through the origin (Fig. 3). Subsequently, the terminal data at higher concentrations where Eq. (6) is valid will tend toward linearity, with a slope equal to the GFR and an extrapolated intercept of k_{tr}. Using the feathering technique one can strip the curve into the components of glomerular filtration and saturable reabsorption as shown in Fig. 4. The saturable reabsorption can also explain the apparent linearity observed at low concentrations when $K_{tr}\ V\ C_u \ll 1$ as Eq. (5) is transformed to Eq. (7) or (8).

$$\begin{aligned}\frac{\Delta X_u}{\Delta t} &= \text{GFR}\ C_u - K_{tr}\ k_{tr}\ V\ C_u \\ &= C_u\ (\text{GFR} - K_{tr}\ k_{tr}\ V)\end{aligned} \tag{7}$$

Since at total concentrations of up to 30 μg/ml, f_u is relatively constant, this linear relationship can also apply to the total drug concentration [Eq. (8)].

$$\frac{\Delta X_u}{\Delta t} = C\ f_u\ (\text{GFR} - K_{tr}\ k_{tr}\ V) \tag{8}$$

For cefixime, despite a twofold increase in f_u (from 10 to 20%), the linear relationship was true for total serum concentrations of up to 100 μg/ml. This suggests the presence of a saturable reabsorption process which, in effect, competes with the concentration-dependent serum protein binding of the drug. Only when f_u is greater than about 20% and the total drug serum concentrations are above 100 μg/ml does the amount of drug filtered at the glomerulus appear to exceed the capacity of the kidney's reabsorption process.

Levy (18) suggested a theoretical model that incorporated the physiological factors influencing renal drug transport. According to the model, if the glomerular filtration rate is proportional to, and the renal secretion rate is a function of, the unbound drug concentration in the serum, then CL_r is a composite of the GFR, tubular secretion, and tubular reabsorption as described below:

$$CL_r = f_u\ \text{GFR} + \frac{Q\ f_u\ k_s}{Q + f_u\ k_s} - F\left(f_u\ \text{GFR} + \frac{Q\ f_u\ k_s}{Q + f_u\ k_s}\right) \tag{9}$$

where k_s is the intrinsic tubular secretion clearance, GFR is the glomerular filtration rate (both clearances are referenced to the free drug concentration in serum), Q is the renal plasma (serum) flow, and F is the fraction of filtered and secreted drug that is reabsorbed. This model assumes that F is constant. When there is no tubular secretion (k_s = O), as

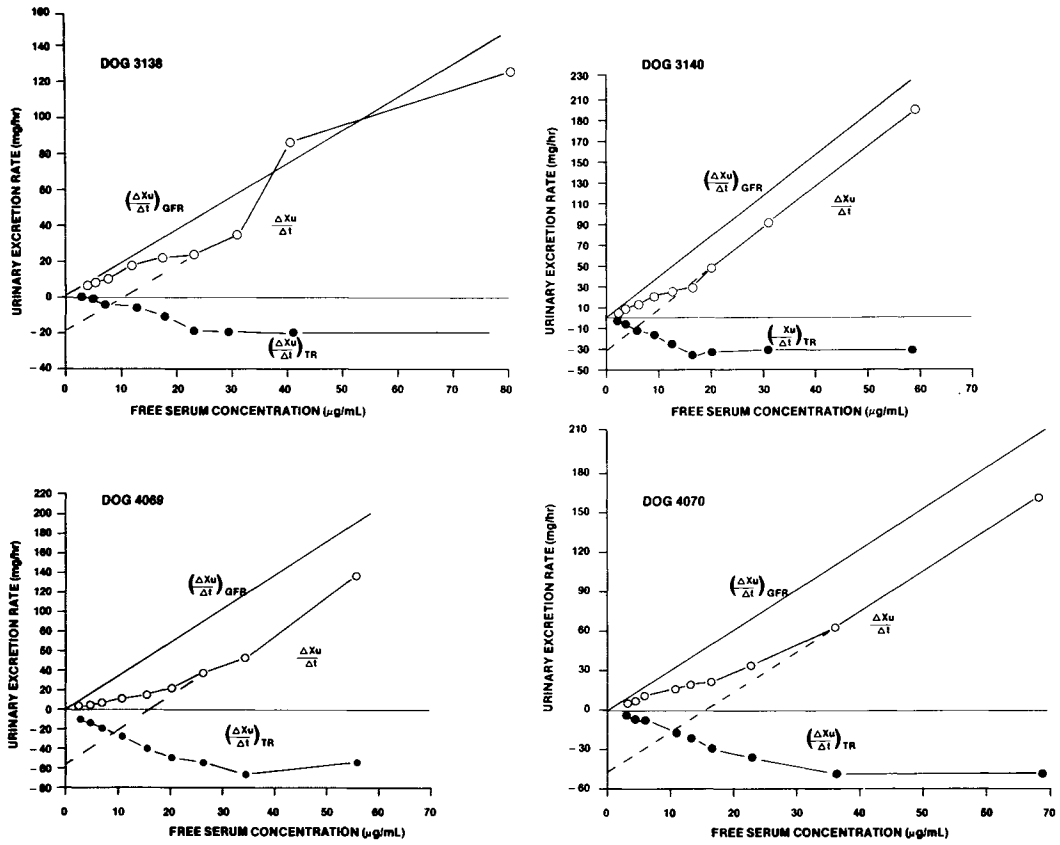

Fig. 4. Urinary excretion versus free serum concentrations of cefixime with extrapolation and feathering according to Eqs. (5) to (8) in individual dogs.

in the case of cefixime in the dog, Eq. (9) can be simplified to Eq. (10).

$$CL_r = f_u\,GFR - F f_u\,GFR = f_u\,GFR\,(1 - F) \quad (10)$$

A plot of CL_r versus f_u should give a straight line which will transect the origin. In this study a plot of CL_r versus f_u for the individual dogs deviated from the relationship of Eq. (10). There was essentially no effect of f_u on CL_r when cefixime was below 15–20% bound. Instead, a constant mean CL_r value of 0.36 ± 0.03 ml/min/kg was observed. As f_u increased above 15 or 20% (depending on the individual dog), a proportional increase in CLr was observed. A plot of CL_r verus f_u in this range (f_u > 20%) gave a straight line for all dogs, with negative Y intercepts (Fig. 5). This can be explained by expanding the theoretical model of Levy (18) to include saturable tubular reabsorption along with saturable protein binding. Under these conditions changes in F occur in addition to changes in f_u. Levy has reported changes in CL_r as a function of f_u, using pooled sulfisoxazole data from individual rats (20). In the current study, changes in CL_r versus f_u were observed in individual dogs as a function of time or concentration.

The results from this study suggests the presence of a saturable tubular reabsorption process for cefixime. At lower serum concentrations, 30–100 μg/ml, the moderate increases in f_u do not generate increases in CL_r because of corresponding increases in tubular reabsorption. This leads to the initial flat region in Fig. 5 (f_u < 20%). As serum concentrations exceed 100 μg/ml there is a continuous increase in f_u but a saturation of reabsorption as it approaches its maximum capacity. In this range, changes in f_u result in corresponding changes in CL_r. Thus, the extensive tubular reabsorption of cefixime may be responsible for the lag in the effect of changing serum drug free fraction on renal clearance. When the amount of drug filtered exceeds the capacity of the active reabsorption process, changes in free fraction will directly affect renal clearance. This is also proposed as the explanation for the negative Y intercept of the CL_r versus f_u plot.

In conclusion, the primary objective of preclinical drug development is to assess the safety and efficacy of potential therapeutic agents. Data generated during these animal tests will assume greater applicability to the clinical situation when reviewed in the context of the kinetics of the compound. Pharmacokinetic support is an integral step in explaining species differences in toxicity, pharmacology, or drug disposition. It is especially relevant for drugs which exhibit nonlinear kinetics—a common occurrence following the administration of unusually high doses mandated by toxicology safety evaluation considerations. Nonlinearities, associated with excessive drug concentrations, can lead to alternate pathways of drug disposition and tissue exposure.

In this study methods were presented which elucidated the serum concentration, protein binding, and renal clearance relationship following a single intravenous dose of the drug. Saturable processes were characterized which offered

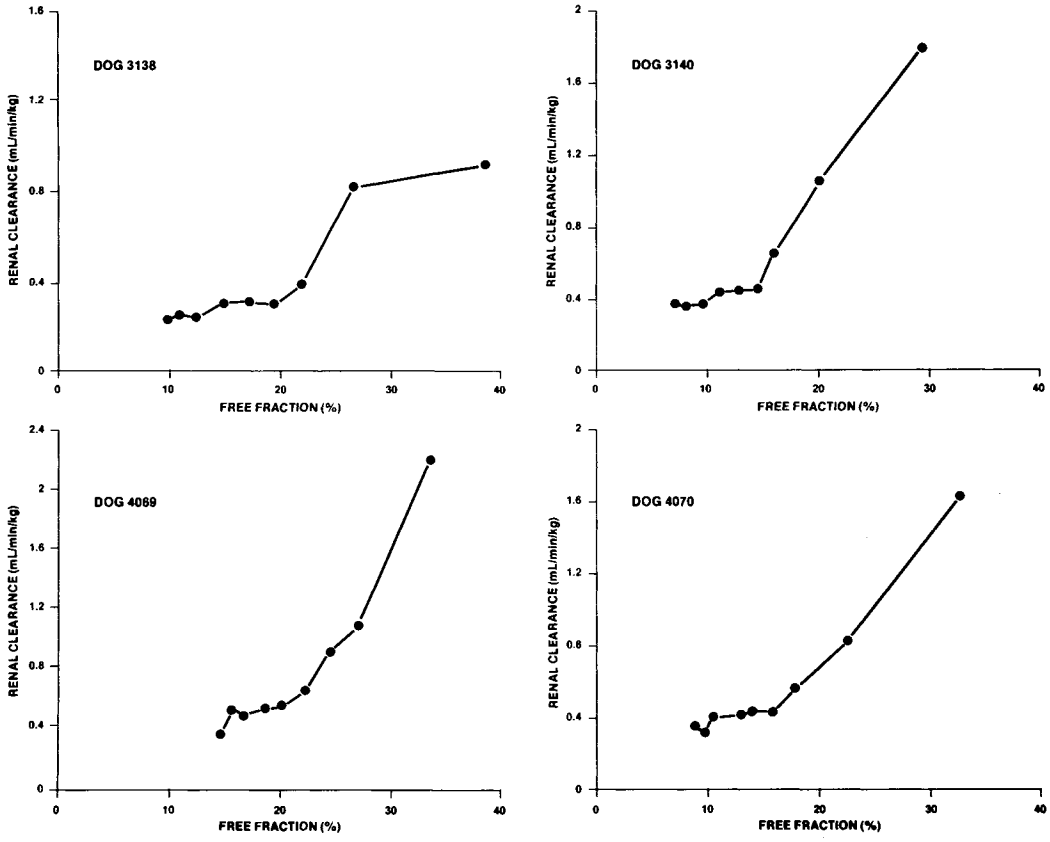

Fig. 5. Renal clearance (CL_r) versus free fraction (f_u) in individual dogs.

an explanation of the nonlinear kinetics of cefixime, previously observed in toxicology safety evaluation and bioavailability studies.

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