Pharmacokinetic modeling of penciclovir and BRL42359 in the plasma and tears of healthy cats to optimize dosage recommendations for oral administration of famciclovir

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OBJECTIVES
To determine, following oral administration of famciclovir, pharmacokinetic (PK) parameters for 2 of its metabolites (penciclovir and BRL42359) in plasma and tears of healthy cats so that famciclovir dosage recommendations for the treatment of herpetic disease can be optimized.

ANIMALS
7 male domestic shorthair cats.

PROCEDURES
In a crossover study, each of 3 doses of famciclovir (30, 40, or 90 mg/kg) was administered every 8 or 12 hours for 3 days. Six cats were randomly assigned to each dosage regimen. Plasma and tear samples were obtained at predetermined times after famciclovir administration. Pharmacokinetic parameters were determined for BRL42359 and penciclovir by compartmental and noncompartmental methods. Pharmacokinetic-pharmacodynamic (PK-PD) indices were determined for penciclovir and compared among all dosage regimens.

RESULTS
Compared with penciclovir concentrations, BRL42359 concentrations were 5- to 11-fold greater in plasma and 4- to 7-fold greater in tears. Pharmacokinetic parameters and PK-PD indices for the 90 mg/kg regimens were superior to those for the 30 and 40 mg/kg regimens, regardless of dosing frequency. Penciclovir concentrations in tears ranged from 18% to 25% of those in plasma. Administration of 30 or 40 mg/kg every 8 hours achieved penciclovir concentrations likely to be therapeutic in plasma but not in tears. Penciclovir concentrations likely to be therapeutic in tears were achieved only with the two 90 mg/kg regimens.

CONCLUSIONS AND CLINICAL RELEVANCE
In cats, famciclovir absorption is variable and its metabolism saturable. Conversion of BRL42359 to penciclovir is rate limiting. The recommended dosage of famciclovir is 90 mg/kg every 12 hours for cats infected with feline herpesvirus. (*Am J Vet Res* 2016;77:833–845)

ABBREVIATIONS

AUC	Area under the concentration-time curve
AUC_{24}	Area under the concentration-time curve from 0 to 24 hours
AUC_{24}:IC_{50}	Ratio of the predicted area under the concentration-time curve from 0 to 24 hours to the half maximal inhibitory concentration
C_{max}	Maximum concentration
FHV-1	Feline herpesvirus type 1
IC_{50}	Half maximal inhibitory concentration
k_a	Absorption rate constant
k_e	Elimination rate constant
k_m	Metabolism rate constant
PD	Pharmacodynamics
PK	Pharmacokinetics
STT	Schirmer tear test
$T > IC_{50}$	Percentage of time the predicted drug concentration remained above a target half maximal inhibitory concentration during each dosing interval
t_{max}	Time to maximum concentration
V_d	Steady-state volume of distribution

Famciclovir is the diacetyl 6-deoxy derivative of penciclovir, a potent antiviral drug that is effective against many herpesviruses, including herpes simplex virus types 1 and 2, varicella-zoster virus, and Epstein-Barr virus.[1] Penciclovir also has favorable efficacy against FHV-1 in vitro, with reported IC_{50}s ranging from 304 to > 3,500 ng/mL.[2–5] In addition, there is mounting evidence that famciclovir is well tolerated and highly effective when administered to cats with experimentally induced herpetic disease[6] or spontaneous disease suspected to be caused by FHV-1.[7–9]

Like acyclovir and ganciclovir, penciclovir is an acyclic guanosine analog that inhibits herpesviral DNA polymerases.[10] However, penciclovir is poorly absorbed following oral administration in humans, and as a result, famciclovir was formulated as an oral prodrug of penciclovir to enhance bioavailability.[11] After oral absorption, the major metabolic pathway for fam-

ciclovir in humans is via di-deacetylation to BRL42359 (an inactive metabolite) followed by oxidation to the active compound, penciclovir.[11] The first deacetylation step occurs predominantly in the intestinal wall, but also occurs to some extent in the blood and liver. The second deacetylation and subsequent oxidation steps occur predominantly in the liver.[10,11] Aldehyde oxidase is the major enzyme involved in the oxidation of BRL42359 to penciclovir.[12,13] There are marked differences among species in the activity of this enzyme in liver cytosol; cats have markedly lower aldehyde oxidase activity, compared with rabbits, monkeys, humans, mice, cows, and dogs.[14,15] In humans,[16,17] dogs, and rats,[18] famciclovir is substantially absorbed and rapidly converted, primarily to penciclovir. Conversely, in cats, famciclovir is incompletely absorbed, and its metabolism to penciclovir becomes saturated at increasing doses, which leads to complex nonlinear PK.[19,20] Penciclovir is present in the tears of cats following oral administration of famciclovir, but its presence in the tears of other species has not been assessed.[19–21] The complex PK profile of penciclovir in cats makes dose recommendations for famciclovir in that species extremely challenging. The goals of the study reported here were to evaluate the PK of famciclovir and its metabolites in the plasma and tears of healthy cats following oral administration of famciclovir at various doses and frequencies and to correlate that in vivo data with reported in vitro antiviral efficacy data to provide a dose recommendation for cats infected with FHV-1.

Materials and Methods

Animals

Seven specific pathogen–free, sexually intact male domestic shorthair cats with a mean ± SD age of 12.5 ± 1.5 months and body weight of 5.47 ± 0.62 kg were used for the study. Each cat was considered healthy on the basis of results of a physical examination, CBC, serum biochemical analysis, and urinalysis. None of the cats had abnormalities detected during an ophthalmic examination. The cats were individually housed in a controlled indoor environment with an ambient temperature that ranged from 20° to 24°C and 14 hours of light and 10 hours of darkness daily. All cats had ad libitum access to fresh water and a commercial dry diet. All study procedures were approved by the University of California-Davis Institutional Animal Care and Use Committee.

Study design

Each of 3 doses of famciclovir (30, 40, or 90 mg/kg) was administered every 8 or 12 hours for 3 consecutive days. Thus, the study had a crossover design with 6 phases. During each phase, 6 cats were randomly assigned to a dosage regimen. There was a washout period of 4 days between each phase, except phases 3 and 4 when a 2-week rest period was observed. The minimum washout period was determined on the basis of pilot data derived from 1 cat that received the maximum famciclovir dosage regimen evaluated in the present study (90 mg/kg, PO, q 8 h for 3 days). Tear and blood samples were collected from that cat daily for 10 days after cessation of famciclovir administration. Beginning 3 days after drug cessation, famciclovir, penciclovir, and BRL42359 concentrations in both plasma and tears were below the limit of quantitation (data not shown). The 2-week rest period between phases 3 and 4 was provided to minimize complications from long-term use of jugular catheters and allow cats time to regenerate blood volume. Sixteen days after the last dose of famciclovir was administered in phase 3, 1 cat developed acute urethral obstruction. On the basis of advice from campus veterinarians, that cat was removed from the study and replaced with another cat for the remaining 3 phases of the study. Thus, 5 cats received all 6 famciclovir dosage regimens and 2 cats received 3 dosage regimens each.

Drug administration and sample collection

Prior to the beginning of each phase, each cat was weighed and the dose of famciclovir required for the assigned regimen was calculated. Commercially available famciclovir tablets[a] were crushed and weighed, and the calculated dose for each cat for that particular phase was packed into gelatin capsules. Capsules were administered with a plastic pill administration device; 5 mL of water was orally administered with a syringe immediately after the capsule was swallowed. Each cat was assigned its own pill administration device and syringe to ensure that it did not receive trace amounts of famciclovir assigned to another cat. All cats were monitored for at least 5 minutes following drug administration to ensure that the capsules were swallowed.

The day before famciclovir administration was initiated for phase 1, each cat was anesthetized for placement of an indwelling 20-gauge, 12-cm, single-lumen catheter[b] in a jugular vein to facilitate blood sample collection. Briefly, each cat was premedicated with atropine (0.02 mg/kg, SC) and butorphanol (0.2 mg/kg SC). Anesthesia was induced with ketamine (5 mg/kg, IV) and midazolam (0.2 mg/kg, IV) and maintained with isoflurane in oxygen during catheter placement. The catheter was removed following sample collection for phase 3 to minimize complications associated with a long-term indwelling catheter. The day before initiation of drug administration for phase 4, the process was repeated to place an identical catheter in the opposite jugular vein for sample collection during the 3 remaining phases.

From each cat during each 3-day period of famciclovir administration, blood and tear samples were collected 3 times daily (immediately prior to administration of the first daily dose [morning trough concentration], immediately prior to administration of the last daily dose [evening trough concentration], and 3 hours after administration of the last daily dose [approx evening peak concentration]). To investigate

the PK after drug cessation, additional blood and tear samples were collected from 3 cats/dosage regimen immediately prior to and at 0.5, 1, 2, 3, 4, 6, 9, and 12 hours after the final dose of each regimen. Following collection of each sample, the blood volume removed was replaced by IV infusion of an equal volume of lactated Ringer solution.

During each phase of the study, approximately 18 to 27 mL of blood was collected from each cat, which represented between 0.45% and 0.67% of the cat's body weight. Numerous safety measures were implemented to minimize the amount and percentage of blood withdrawn from each cat. Only cats with a body weight > 4 kg were enrolled in the study. For each cat, the PCV was monitored at the end of each phase, and if the PCV was < 20%, blood collection was terminated. Finally, the cats were allowed a 2-week rest period midway through the study to allow them time to regenerate blood volume.

Blood samples were collected into evacuated glass tubes that contained lithium heparin and centrifuged at 1,240 × *g* for 5 minutes. Immediately after centrifugation, plasma was harvested from each sample and stored at –80°C until analysis. Tear samples were collected from both eyes with plain STT strips[c] as described.[21] Prior to use, each STT strip was placed in an individual cryovial, and the baseline mass of the dry strip within its cryovial was obtained. Each STT strip was then placed in the ventrolateral conjunctival fornix of 1 eye with clean forceps, left for approximately 1 minute, removed, and immediately replaced into its cryovial and reweighed. The difference in mass before and after tear collection was used to estimate the volume of tears collected; it was assumed that 1 g of tears was equivalent to 1 mL. Following determination of the post-tear collection mass, each STT strip was suspended in 2 mL of methanol and stored at –80°C until analysis.

Sample analysis

Analytical reference standards were obtained for penciclovir,[d] BRL42359,[d] and famciclovir,[e] and an internal standard was obtained for acyclovir.[f] Stock solutions (1 mg/mL) of each reference solution were prepared in methanol (famciclovir and BRL42359) or methanol and water (penciclovir and acyclovir). All solvents were high-performance–liquid-chromatography grade or better.

Samples were analyzed with liquid chromatography–tandem mass spectrometry. Analysis was conducted as described for famciclovir, penciclovir, and BRL42359 concentrations in plasma[19,22] and for penciclovir and famciclovir concentrations in tears.[21] For analysis of tear BRL42359 concentrations, tear calibrators of BRL42359 were prepared by dilution of standard solutions with methanol to 0.05, 0.1, 0.3, 0.5, 1, 2, 5, 10, 25, and 50 ng/mL. Calibration curves and negative control samples were freshly prepared for each quantitative assay. Quality control samples (methanol fortified with the analyte of interest at 3 concentrations within the standard curve) were included with each sample set. A 1-mL aliquot of each sample was mixed with 100 µL of methanol that contained acyclovir (250 ng/mL), vortexed gently, dried under nitrogen at 50°C, and then dissolved in 150 µL of 5% acetonitrile in water, both with 0.2% formic acid. The injection volume was 30 µL. The detection and quantification methodology for tears was the same as that described for plasma,[19,22] except the initial acetonitrile concentration was held at 0% for 0.33 minutes, increased to 70% over 3.34 minutes, and then increased to 90% over 0.33 minutes before reequilibrating for 3.17 minutes at the initial conditions. Quadratic curves were used to quantitate BRL42359 from 0.05 to 50 ng/mL. A weighting factor of 1/X was used for calibration curves, all of which had correlation coefficients ≥ 0.99. Interday precision and accuracy were determined with quality control samples in replicates of 6. The limit of quantitation was 0.05 ng/mL.

PK analysis

Noncompartmental PK analyses of plasma and tear penciclovir and BRL42359 concentrations were performed by the use of commercial software,[g] and data collected after administration of the final dose of each of the 6 dosage regimens. Pharmacokinetic parameters were determined with standard noncompartmental equations. Terminal half-life was calculated as 0.693/λz, where λz is the slope of the terminal phase.

Commercially available software[h] was used to develop a compartmental PK model to describe plasma and tear penciclovir and BRL42359 concentrations. Famciclovir was not detected in the plasma at any time; therefore, the model did not include any components to describe the PK of famciclovir. Famciclovir was assumed to be completely and instantaneously converted to BRL42359 after administration by use of a molar mass ratio of 0.734.[23] Absorption of BRL42359 after oral administration was described by first-order transfer with an absorption rate constant. That hybrid rate constant included the extravascular and intravascular metabolism of famciclovir to BRL42359 and the transfer of both species from absorption to central compartments. BRL42359 disposition was described by a 1-compartment model. Because IV data for BRL42359 were not available, the V_d reported was corrected for bioavailability (ie, V_d/F).[24] BRL42359 was eliminated from the central compartment by means of a first-order process. Conversion of BRL42359 to penciclovir was modeled with a first-order process driven by BRL42359 concentrations by use of a molar mass ratio 0.93.[23]

A 2-compartment model was chosen for penciclovir on the basis of the PK model for penciclovir after IV administration.[20] Because the observed penciclovir concentrations were dependent on the unknown proportion of BRL42359 that was converted to penciclovir, the V_d for penciclovir could not be

identified on the basis of the observed concentrations alone. Therefore, the apparent V_d for penciclovir was fixed for all individuals at 0.6 L/kg.[20] Penciclovir was eliminated from the central compartment only by first-order elimination. For both BRL42359 and penciclovir, the steady-state definition of V_d was used.[24]

Tear concentrations of BRL42359 and penciclovir were described with volumeless compartments. Proportionality between plasma and tear concentrations was assumed; therefore, the concentration of a given metabolite in those compartments was modeled as a function of the predicted plasma concentration of that metabolite at the specified time. The partition coefficient parameter was estimated separately for both metabolites, and that process did not include a lag time. Estimation of tear parameters was performed after plasma parameters had been estimated, so the tear data had no effect on plasma parameter estimates. To optimize the model, nonlinear regression and direct-search methods were used, with weighted sum-of-squares of residuals as the objective function. The goodness-of-fit for the models was assessed by visual examination of plots of observed and predicted data and plots of weighted residuals. The final parameters reported were obtained by regression at the level of study phase.

PK-PD indices

The 2 in vitro IC_{50}s of penciclovir against FHV-1 chosen for PK-PD evaluation were 304 ng/mL, which is the lowest IC_{50} reported,[3] and 860 ng/mL,[2] which is the IC_{50} previously calculated for the same viral isolate in the only prospective, placebo-controlled famciclovir efficacy trial[6] to date. Other reported IC_{50}s were not considered because they have been reported to be erroneous (3,500 ng/mL)[2,4] or inconsistent with the observed clinical effectiveness of famciclovir in cats.[4,5] The $T > IC_{50}$ and AUC_{0-24}:IC_{50} were calculated at the presumed steady state by use of the 2 selected IC_{50}s and in vivo data collected during the present study.

Statistical analysis

Mixed-effects linear regression models were applied to the estimated primary PK parameters, secondary PK parameters, and PK-PD indices. Separate models were created to analyze dose magnitude, administration frequency, and each dosage regimen (referent was the 90 mg/kg-every-8-hours regimen; ie, phase was controlled) as categorical fixed effects, with cat identification treated as a random effect. The goodness-of-fit of each linear model was assessed by residual analysis. All analyses were performed by commercial statistical software,[i] and values of $P < 0.05$ were considered significant.

Results

Cats

All cats except the one that developed acute urethral obstruction remained healthy throughout the study. The PCV for all cats remained > 20% for the duration of the study, and no notable alterations in body weight, temperature, heart rate, respiratory rate, or CBC and serum biochemical variables were detected during the observation period for any cat.

The cat that developed acute urethral obstruction was euthanized with IV administration of pentobarbital sodium and phenytoin sodium solution on the basis of recommendations by the campus veterinarians. That cat received famciclovir at 90 mg/kg every 12 hours for 3 days during phase 1, 90 mg/kg every 8 hours for 3 days during phase 2, and 40 mg/kg every 12 hours for 3 days during phase 3. Postmortem examination of the cat revealed bilateral renal swelling, a blood clot in the pelvis of 1 kidney, and intraluminal hemorrhages in the urinary bladder; the other organs appeared grossly normal. The underlying cause of the urethral obstruction was not determined. Serum creatinine (13 mg/dL; reference range, 1.1 to 2.2 mg/dL) and BUN (204 mg/dL; reference range, 18 to 33 mg/dL) concentrations were both abnormally increased at the time of illness; however, the serum creatinine concentration (1.0 mg/dL) at the end of phase 3 (16 days prior to onset of illness) was the same as that measured before study initiation and just below the reference range.

Sample analysis

The accuracy (as a percentage of the nominal concentration) and precision (as a percentage of the relative SD) of the liquid chromatography–tandem mass spectrometry assay for famciclovir, penciclovir, and BRL42359 in plasma and tears were summarized **(Appendix 1)**. Famciclovir was detected in only 3 of 477 plasma samples and only in samples that were collected ≤ 1 hour following administration. The famciclovir concentration was ≤ 15 ng/mL for all 3 samples. Consequently, insufficient data were available for determination of PK parameters for famciclovir in plasma. By contrast, BRL42359 and penciclovir were detected in plasma samples collected at all time points except baseline. Plasma and tear penciclovir and BRL42359 concentrations **(Figure 1)** followed a similar pattern; however, when all dosage regimens were considered, mean plasma BRL42359 concentrations were 5- to 11-fold higher than mean plasma penciclovir concentrations. Famciclovir, BRL42359, and penciclovir were present in all tear samples. Mean tear BRL42359 concentrations were 4- to 7-fold higher than mean tear penciclovir concentrations. Dependent on the dosage regimen administered, tear penciclovir concentrations ranged from 18% to 25% of the corresponding plasma penciclovir concentrations.

PK model

Examination of model predictions and residuals revealed that a 1-compartment model best described the PK of BRL42359, whereas a 2-compartment model best described the PK of penciclovir. For both models, residuals approximated normal distributions **(Appendix 2)**, and plots of the predicted versus estimated metabolite concentrations were approxi-

mately linear **(Appendix 3)**. The PK parameters for BRL42359 **(Table 1)** and penciclovir **(Table 2)** in plasma and tears derived from compartmental and noncompartmental analyses were summarized. Although BRL42359 does not directly contribute to the therapeutic effect of famciclovir, the PK parameters for BRL42359 in plasma and tears contributed to the understanding of the PK parameters for penciclovir and were provided for completeness.

The coefficient of variation was < 51% for all parameters, except for the k_a for famciclovir, which had a coefficient of variation of 108%. In 5 of the detailed data series obtained from 3 cats, the k_a could not be confidently estimated because it appeared that the t_{max} for BRL42359 in those cats occurred before the first sampling point (30 minutes after drug administration). However, for the other data series, estimates of k_a were at least equal to, and generally exceeded, the k_e for BRL42359 and penciclovir. The mean k_a did not differ significantly between regimens in which the famciclovir was administered every 8 hours (8-hour regimens) and those in which the famciclovir was administered every 12 hours (12-hour regimens; $P = 0.83$) or between regimens in which the 90 mg/kg dose was administered (ie, 90 mg/kg regimens) and regimens in which the 30 mg/kg (30 mg/kg regimens; $P = 0.11$) or 40 mg/kg (40 mg/kg regimens; $P = 0.68$) dose was administered. The mean k_m did not differ significantly ($P = 0.73$) between the 8-hour regimens and 12-hour regimens; however, the mean k_m for the 90 mg/kg regimens was significantly less than the mean k_m for the 30 mg/kg ($P < 0.001$) or 40 mg/kg ($P = 0.02$) regimens, which suggested that there was a saturable process in the metabolism of famciclovir to penciclovir at higher doses. The mean k_m for the 30 and 40 mg/kg regimens was 2- and 1.5-fold, respectively, greater than the mean k_m for the 90 mg/kg regimens. The mean k_m did not differ significantly ($P = 0.29$ to 1.00) among the 6 study phases.

The mean AUC_{24} and C_{max} for penciclovir in plasma for the 90 mg/kg regimens were significantly ($P < 0.001$) greater than those for the 30 and 40 mg/kg regimens regardless of dosing frequency (ie, when dosing frequency was not controlled in the model).

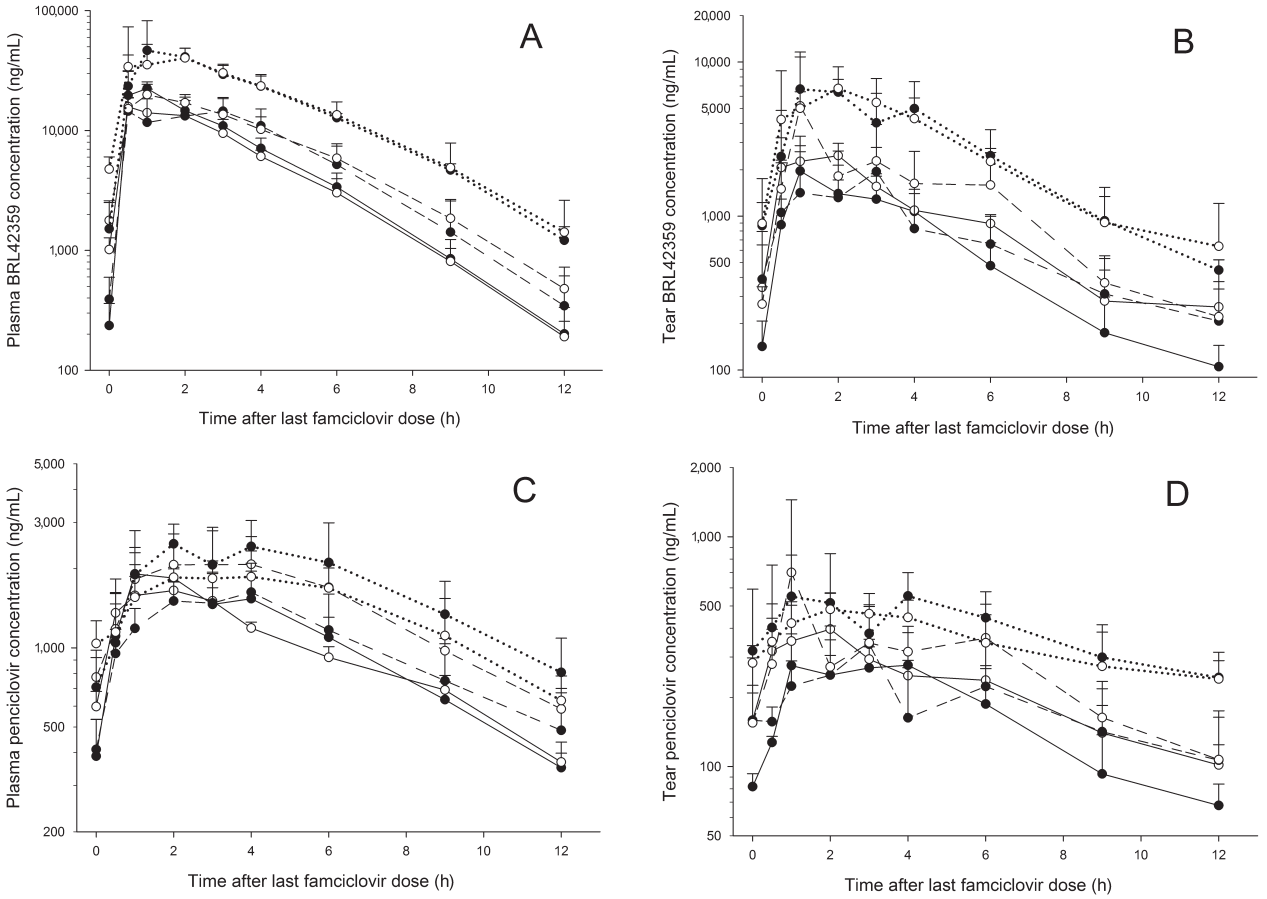

Figure 1—Mean ± SD concentration of BRL42359 (A and B) and penciclovir (C and D) in the plasma (A and C) and tears (B and D) of 6 healthy sexually intact male domestic shorthair cats over time following oral administration of famciclovir at each of 3 doses (30 mg/kg [solid line], 40 mg/kg [dashed line], and 90 mg/kg [dotted line]) for each of 2 dosing intervals (every 8 hours [white circles] and every 12 hours [black circles]) for 3 consecutive days. The study had a crossover design with 6 phases. During each phase, 6 cats were randomly assigned to a dosage regimen. There was a washout period of 4 days between each phase, except phases 3 and 4 when a 2-week rest period was observed. One cat developed acute urethral obstruction during the rest period between phases 3 and 4 and was replaced with another cat for the remainder of the study. Thus, 5 cats received all 6 dosage regimens and 2 cats each received 3 dosage regimens.

Table 1—Pharmacokinetic parameters for BRL42359 in the plasma and tears of 6 healthy sexually intact male domestic shorthair cats following oral administration of famciclovir at each of 6 dosage regimens.

		Dosage regimen						
Parameter	**Sample type**	**30 mg/kg q 12 h**	**40 mg/kg q 12 h**	**90 mg/kg q 12 h**	**30 mg/kg q 8 h**	**40 mg/kg q 8 h**	**90 mg/kg q 8 h**	**All regimens combined**
k_a (h^{-1})	Plasma	37.6 (84.3)	16.4 (130)	23.3 (130)	26.4 (81.4)	32.1 (104)	13.3 (187)	24.8 (108)
k_m (h^{-1})	Plasma	0.037 (44.1)	0.024 (28.6)	0.014 (51.4)	0.027 (36.9)	0.023 (36.8)	0.018 (59.9)	0.024 (50.9)
k_e (h^{-1})	Plasma	0.376 (7.86)	0.474 (30.2)	0.347 (22.0)	0.402 (19.1)	0.368 (16.2)	0.456 (27.4)	0.404 (24.4)
$V_{d(ss)}$/F (L/kg)	Plasma	0.928 (28.3)	1.03 (46.4)	1.32 (33.7)	0.921 (37.1)	0.942 (33.9)	1.08 (31.0)	1.04 (35.9)
P–T	Plasma	0.104 (43.5)	0.119 (46.6)	0.138 (37.8)	0.117 (32.3)	0.0766 (45.6)	0.139 (24.0)	0.116 (39.9)
C_{max} (ng/mL)*	Plasma	26,125 ± 930	23,765 ± 9,125	56,150 ± 22,080	17,940 ± 1,025	21,105 ± 3,070	55,330 ± 21,745	—
	Tears	1,960 ± 655	2,580 ± 680	8,775 ± 2,290	2,620 ± 640	5,300 ± 5,515	8,430 ± 845	—
$C_{(ave)}$ (ng/mL)*	Plasma	6,220 ± 680	6,600 ± 375	16,940 ± 4,850	7,280 ± 670	10,485 ± 1,545	23,155 ± 1,575	—
	Tears	675 ± 125	755 ± 240	2,830 ± 600	1,345 ± 135	1,900 ± 1,250	3,830 ± 825	—
$C_{ss(min)}$ (ng/mL)*	Plasma	195 ± 135	260 ± 135	880 ± 395	965 ± 375	1,700 ± 880	3,970 ± 925	—
	Tears	85 ± 20	130 ± 60	395 ± 75	345 ± 305	270 ± 80	895 ± 330	—
t_{max} (h)*	Plasma	0.7 ± 0.3	2.2 ± 1.4	1.3 ± 0.6	1.2 ± 0.8	1.5 ± 0.6	1.5 ± 0.9	—
	Tears	1.0 ± 0.0	2.3 ± 1.2	2.0 ± 1.7	1.2 ± 0.8	1.5 ± 1.0	1.8 ± 1.3	—
$t_{1/2(z)}$ (h)*	Plasma	1.4 ± 0.3	1.5 ± 0.2	1.8 ± 0.3	1.5 ± 0.2	1.6 ± 0.2	1.8 ± 0.6	—
	Tears	2.3 ± 0.6	2.9 ± 0.6	2.5 ± 0.0	2.8 ± 1.1	2.5 ± 1.0	2.7 ± 0.7	—

Parameters were derived from compartmental or noncompartmental analysis. Values represent the mean (coefficient of variation) or mean ± SD. Each of 3 doses of famciclovir (30, 40, or 90 mg/kg) was administered every 8 or 12 hours for 3 consecutive days. The study had a crossover design with 6 phases. During each phase, 6 cats were randomly assigned to a dosage regimen. There was a washout period of 4 days between each phase, except phases 3 and 4 when a 2-week rest period was observed. One cat developed acute urethral obstruction during the rest period between phases 3 and 4 and was replaced with another cat for the remainder of the study. Thus, 5 cats received all 6 famciclovir dosage regimens and 2 cats received 3 dosage regimens each.

*Derived from noncompartmental analysis. — = Not determined. $C_{(ave)}$ = Mean drug concentration during the dosing interval at steady-state. $C_{ss(min)}$ = Observed minimum drug concentration during the dosing interval at steady-state. F = Bioavailability. P–T = Plasma to tear partition coefficient. $t_{1/2(z)}$ = Apparent elimination half-life. $V_{d(ss)}$ = Apparent volume of distribution at steady-state.

Table 2—Pharmacokinetic parameters for penciclovir in the plasma and tears of the cats in Table 1.

		Dosage regimen						
Parameter	**Sample type**	**30 mg/kg q 12 h**	**40 mg/kg q 12 h**	**90 mg/kg q 12 h**	**30 mg/kg q 8 h**	**40 mg/kg q 8 h**	**90 mg/kg q 8 h**	**All regimens combined**
k_e (h^{-1})	Plasma	0.710 (21.2)	0.631 (34.2)	0.615 (34.6)	0.584 (24.4)	0.632 (43.5)	0.702 (43.3)	0.646 (33.0)
k_{12} (h^{-1})	Plasma	1.20 (17.7)	1.20 (27.7)	1.08 (13.6)	0.955 (23.0)	0.872 (54.7)	1.19 (17.3)	1.08 (27.4)
k_{21} (h^{-1})	Plasma	1.01 (20.5)	0.897 (47.8)	0.948 (38.1)	0.932 (35.9)	0.846 (45.2)	1.16 (18.4)	0.965 (33.5)
$V_{d(ss)}$ (L/kg)*	Plasma	0.6 (0)	0.6 (0)	0.6 (0)	0.6 (0)	0.6 (0)	0.6 (0)	0.6 (0)
P–T	Plasma	0.153 (33.1)	0.216 (30.8)	0.216 (27.2)	0.180 (31.0)	0.172 (29.6)	0.234 (26.6)	0.195 (31.2)
AUCτ (h•ng/mL)	Plasma	10,340 ± 1,745	10,685 ± 1,705	16,550 ± 3,665	7,335 ± 1,555	8,435 ± 1,770	10,385 ± 2,315	—
	Tears	1,585 ± 560	2,320 ± 890	3,605 ± 1,400	1,290 ± 355	1,420 ± 440	2,470 ± 1,055	—
AUC_{0-24} (h•ng/mL)	Plasma	20,675 ± 3,490	21,370 ± 3,410	33,100 ± 7,330	22,005 ± 4,670	25,305 ± 5,310	31,160 ± 6,950	—
	Tears	3,165 ± 1,115	4,640 ± 1,780	7,215 ± 2,805	3,870 ± 1,060	4,260 ± 1,330	7,415 ± 3,170	—
Dose-corrected AUC_{0-24} (h•ng/mL)	Plasma	690 ± 115	535 ± 85	370 ± 80	735 ± 155	635 ± 135	345 ± 80	—
	Tears	105 ± 35	115 ± 45	80 ± 30	105 ± 35	105 ± 35	80 ± 35	—
C_{max} (ng/mL)†	Plasma	2,010 ± 330	1,945 ± 695	2,720 ± 655	1,755 ± 170	2,210 ± 600	2,015 ± 165	—
	Tears	305 ± 80	375 ± 120	680 ± 220	395 ± 170	750 ± 720	555 ± 30	—
$C_{(ave)}$ (ng/mL)†	Plasma	1,105 ± 230	1,100 ± 350	1,785 ± 525	1,195 ± 20	1,750 ± 400	1,640 ± 105	—
	Tears	170 ± 25	190 ± 70	400 ± 120	270 ± 70	345 ± 130	395 ± 50	—
$C_{ss(min)}$ (ng/mL)†	Plasma	345 ± 100	445 ± 145	630 ± 250	570 ± 65	790 ± 260	905 ± 255	—
	Tears	65 ± 15	55 ± 15	200 ± 70	160 ± 65	150 ± 50	275 ± 60	—
t_{max} (h)†	Plasma	1.3 ± 0.6	2.3 ± 1.2	2.7 ± 0.6	1.7 ± 0.6	2.5 ± 0.6	2.7 ± 1.6	—
	Tears	4.7 ± 1.2	3.3 ± 0.6	2.3 ± 1.5	2.0 ± 0.0	2.6 ± 2.5	3.0 ± 1.0	—
$t_{1/2(z)}$ (h)†	Plasma	3.6 ± 0.2	4.7 ± 1.1	4.7 ± 1.2	4.7 ± 0.7	4.1 ± 0.7	4.4 ± 1.0	—
	Tears	4.3 ± 0.8	5.9 ± 1.5	6.9 ± 0.9	5.1 ± 2.0	3.8 ± 1.4	10.4 ± 3.8	—

*$V_{d(ss)}$ was held constant at 0.6 for all dosage regimens on the basis of results of a previous study.[20] †Derived from noncompartmental analysis.

AUC_τ = Area under the plasma concentration-time curve during the dosing interval. k_{12} = First-order rate constant for transfer of unbound drug between the central and peripheral compartments. k_{21} = First-order rate constant for the transfer of unbound drug between the peripheral and central compartments.

See Table 1 for remainder of key.

Conversely, the mean dose-corrected AUC_{24} in plasma for the 90 mg/kg regimens was significantly ($P < 0.001$) less than that for the 30 and 40 mg/kg regimens. The mean dose-corrected AUC_{24} for penciclovir in plasma did not differ significantly ($P = 1.00$) among the 6 study phases, which indicated that penciclovir exposure did not change among the phases. The mean AUC_{24} ($P = 0.47$) and C_{max} ($P = 0.21$) did not

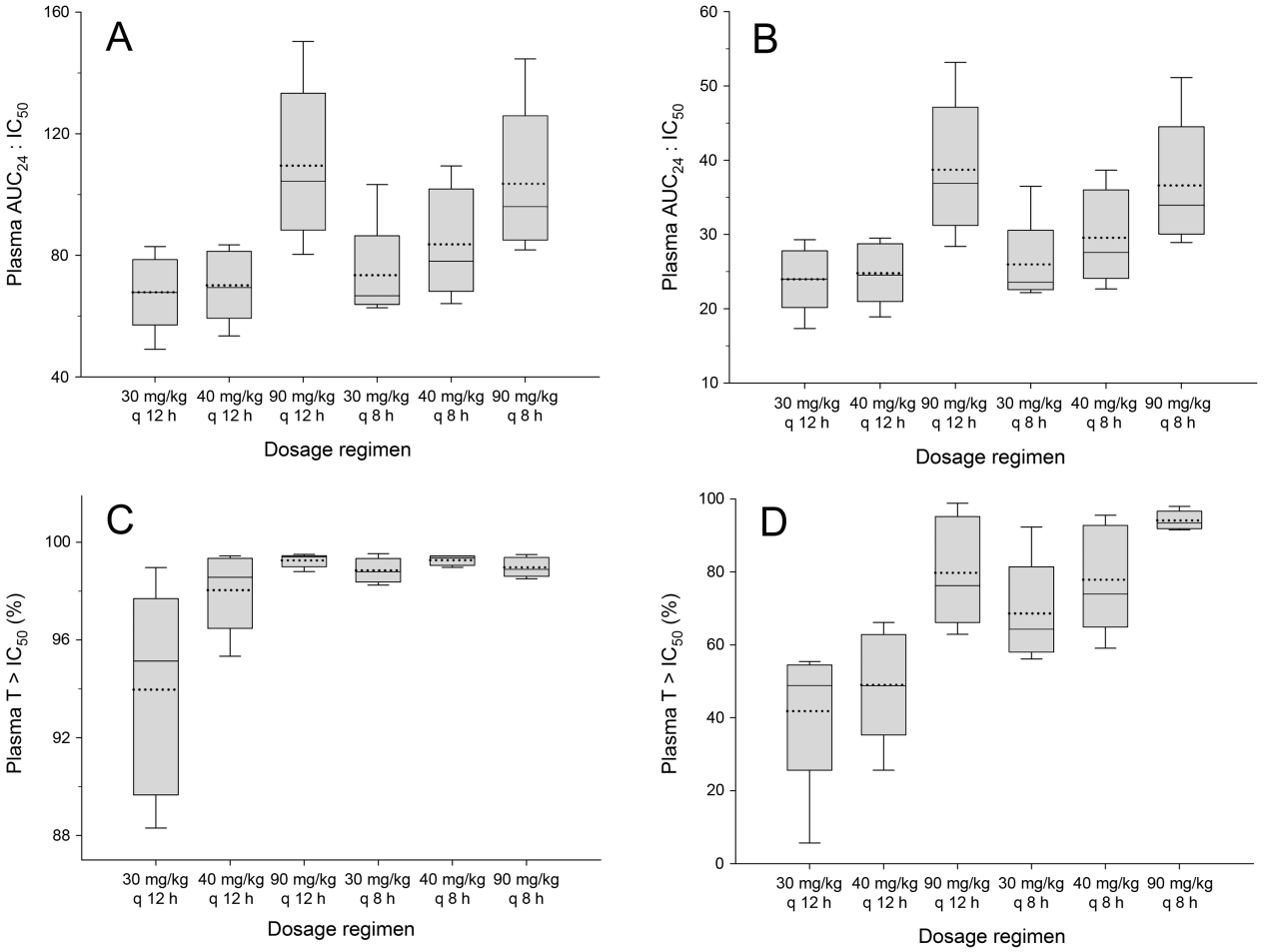

Figure 2—Box-and-whisker plots of the AUC_{24}:IC_{50} (A and B) and T > IC_{50} (C and D) for penciclovir in the plasma of the cats of Figure 1. Target IC_{50}s used for the calculations were 304 ng/mL (A and C) and 860 ng/mL (B and D). For each plot, the bottom and top of the box delimit the 25th and 75th percentiles, respectively, the solid and dotted lines within the box represent the median and mean, respectively, and the whiskers delimit the range. ***See*** Figure 1 for remainder of key.

differ significantly between the 8-hour regimens and 12-hour regimens regardless of the dose of famciclovir administered (ie, when dose was not controlled in the model). The mean AUC_{24} and C_{max} for penciclovir in plasma for the 90 mg/kg-every-8-hours regimen did not differ significantly from the mean AUC_{24} ($P = 0.17$) and C_{max} ($P = 0.84$) for the 90 mg/kg-every-12-hours regimen but were significantly ($P \leq 0.001$ for all comparisons) greater than those for all other regimens evaluated. The mean AUC_{24} for penciclovir in tears for the 90 mg/kg regimens was significantly ($P < 0.001$) greater than that for the 30 and 40 mg/kg regimens. However, the mean AUC_{24} for penciclovir in tears did not differ significantly ($P = 0.39$) between the 8-hour and 12-hour regimens.

PK-PD indices for penciclovir in plasma

The PK-PD indices for penciclovir in plasma were summarized **(Figure 2)**. For both target IC_{50}s evaluated (304 and 860 ng/mL), the mean T > IC_{50} and AUC_{24}:IC_{50} in plasma for the 90 mg/kg regimens were significantly ($P \leq 0.001$) greater than those for the 30 and 40 mg/kg regimens regardless of the frequency of famciclovir administration. For both IC_{50}s evaluated, the mean T > IC_{50} in plasma for the 8-hour regimens was significantly ($P \leq 0.02$) greater than that for the 12-hour regimens; however, the mean AUC_{24}:IC_{50} did not differ significantly ($P = 0.47$) between the 8- and 12-hour regimens. Specifically, the mean T > IC_{50} in plasma for the 90 mg/kg-every-8-hours regimen was significantly ($P = 0.001$) greater than that for the 90 mg/kg-every-12-hours regimen when the target IC_{50} used was 860 ng/mL, but the mean T > IC_{50} in plasma did not differ significantly ($P = 0.74$) between those 2 regimens when the target IC_{50} used was 304 ng/mL.

When the target IC_{50} used was 304 ng/mL, the mean T > IC_{50} for penciclovir in plasma exceeded 94% of the dosing interval for all 6 dosage regimens. The mean T > IC_{50} in plasma for the 90 mg/kg-every-8-hours regimen was significantly ($P < 0.001$) greater than that for the 30 mg/kg-every-12-hours regimen but did not differ significantly from that for any of the other regimens evaluated.

When the target IC_{50} used was 860 ng/mL, the

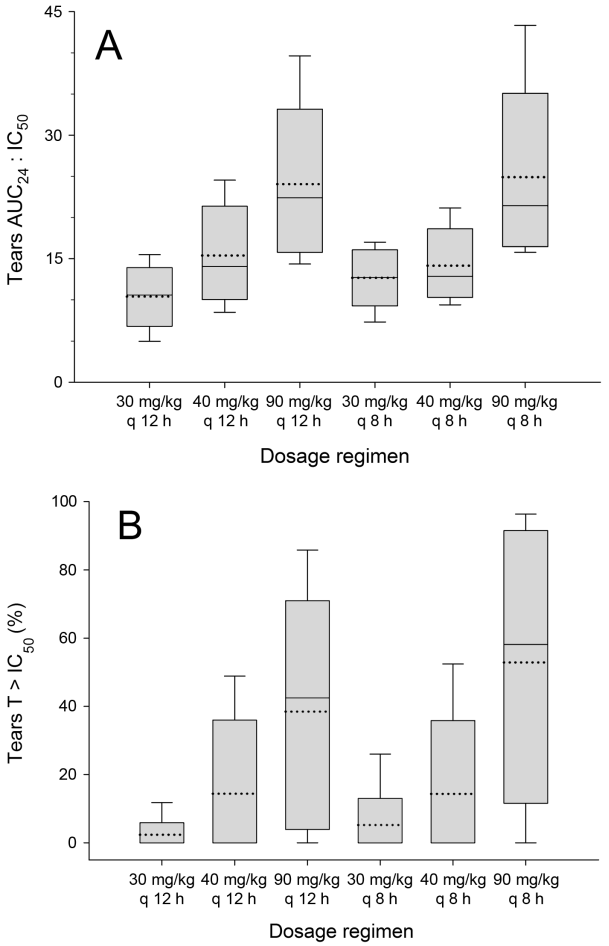

Figure 3—Box-and-whisker plots of the AUC_{24}:IC_{50} (A) and T > IC_{50} (B) for penciclovir in the tears of the cats of Figure 1. The target IC_{50} used for the calculations was 304 ng/mL. ***See*** Figures 1 and 2 for remainder of key.

mean T > IC_{50} in plasma varied between 43.1% and 94.0% of the dosing interval. The mean T > IC_{50} in plasma for the 90 mg/kg-every-8-hours regimen was significantly ($P \leq 0.001$) greater than that for all other dosage regimens.

The mean AUC_{24}:IC_{50} in plasma varied from 68.0 to 108.9 when the target IC_{50} used was 304 ng/mL and from 24.0 to 38.5 when the target IC_{50} used was 860 ng/mL. Regardless of the target IC_{50} used, the mean AUC_{24}:IC_{50} in plasma for the 90 mg/kg-every-8-hours regimen was significantly ($P \leq 0.001$) greater than that for all other dosage regimens except the 90 mg/kg-every-12-hours regimen ($P = 0.17$).

PK-PD indices for penciclovir in tears

The PK-PD indices for penciclovir in tears were summarized **(Figure 3)**. Tear penciclovir concentrations never exceeded the target concentration of 860 ng/mL for any of the 6 dosage regimens; therefore, only an IC_{50} of 304 ng/mL was used to evaluate the T > IC_{50} in tears. The mean T > IC_{50} and AUC_{24}:IC_{50} in tears for the 90 mg/kg regimens were significantly ($P < 0.001$) greater than those for the 30 and 40 mg/kg regimens regardless of frequency of famciclovir administration. However, the mean T > IC_{50} ($P = 0.23$) and AUC_{24}:IC_{50} ($P = 0.39$) did not differ significantly between the 8- and 12-hour regimens regardless of the dose. The mean T > IC_{50} for penciclovir in tears ranged from 2.0% to 53.4% of dosing interval. The mean T > IC_{50} in tears for the 90 mg/kg-every-8-hours regimen was significantly ($P \leq 0.001$) greater than that for all other dosage regimens, except the 90 mg/kg-every-12-hours regimen ($P = 0.054$).

The mean AUC_{24}:IC_{50} in tears for all 6 dosage regimens ranged from 10.4 to 24.4 when the target IC_{50} used was 304 ng/mL and from 3.7 to 8.6 when the target IC_{50} used was 860 ng/mL. Regardless of the target IC_{50} used, the mean AUC_{24}:IC_{50} in tears for the 90 mg/kg-every-8-hours regimen was significantly ($P < 0.001$) greater than that for all other dosage regimens except the 90 mg/kg-every-12-hours regimen ($P = 0.62$).

Discussion

In the present study, orally administered famciclovir was readily absorbed and rapidly converted to BRL42359 in healthy cats. Furthermore, results indicated that the subsequent conversion of BRL42359 to penciclovir was the rate-limiting step in the metabolism of famciclovir and the likely cause of the nonlinear PK of famciclovir in cats.

Although famciclovir absorption in the cats of the present study was adequate to achieve targeted penciclovir concentrations in both plasma and tears, it varied greatly. This was most likely caused (at least in part) by the absence of critical data from the model we developed because the earliest sampling point (30 minutes after famciclovir administration) did not provide sufficient observations during the absorption phase in all cats. Additionally, conversion of famciclovir to BRL42359 was assumed to be and modeled as instantaneous transfer, and error associated with that assumption may have affected the estimated absorption rate. Moreover, the cats of the present study had ad libitum access to food, and the extent and rate of famciclovir absorption may have been negatively affected by recent food intake as it is in humans.[25] Finally, it is possible that famciclovir absorption was nonlinear because of saturation of an active famciclovir transporter in the intestinal wall, a mechanism that has been proposed for the ganciclovir prodrug, valganciclovir.[26] Rapid metabolism and potentially complex absorption may limit the value of further assessment of famciclovir absorption by in vivo PK trials.

Famciclovir was detected at very low concentrations (≤ 15 ng/mL) in only 3 plasma samples collected ≤ 1 hour after administration. This suggested that, in cats, famciclovir likely undergoes substantial first-pass metabolism following absorption. In contrast, famciclovir was detected in most of the tear samples analyzed. The discrepancy in the incidence of famciclovir detection between plasma and tears is intriguing and warrants further investigation. It is possible that distribution of famciclovir to the tear compartment

is very rapid, or that the rate at which famciclovir is metabolized to BRL42359 differed for blood samples that were collected into glass tubes that contained lithium heparin as an anticoagulant and tear samples that were collected by STT strips. Alternately, famciclovir may be retained in the tear film such that its rate of elimination is slower than the rate of uptake from plasma. The pattern of residuals for tear concentration estimates of BRL42359 indicated that these data were predicted more poorly by the model than were the penciclovir concentrations (Appendix 2). Neither the plasma-proportionate model nor any other currently available model is suitable to describe the PK of BRL42359, and further evaluation of the PK of BRL42359 was beyond the scope of the present study.

High concentrations of BRL42359 were detected in both the plasma and tears of the cats of the present study, which suggested that BRL42359 is the major intermediate metabolite in the conversion of famciclovir to penciclovir in cats as it is in other species.[16,18] Dideacetylation of famciclovir to form BRL42359 is rapid and complete. The subsequent oxidation of BRL42359 to penciclovir is rate limiting and the likely cause of the nonlinear and inefficient metabolism of famciclovir in cats. In the present study, the dose-corrected penciclovir exposure decreased as the dose of famciclovir increased, as evidenced by the fact that the k_m for the 90 mg/kg regimens was 2-fold less than the k_m for the 30 mg/kg regimens and 1.5-fold less than the k_m for the 40 mg/kg regimens. Collectively, those observations supported a saturable mechanism for the conversion of BRL42359 to penciclovir. This is most likely attributable to the fact that hepatic aldehyde oxidase, an enzyme critical for metabolism of BRL42359, has limited activity in cats, compared with its activity in other species.[12,13] Elucidation of that process will require in vitro metabolism studies or further in vivo models.[27] BRL423596 has very low cytotoxicity in vitro, which is fortunate given the high plasma BRL42359 concentrations that accumulated in the cats of the present study following famciclovir administration; however, it also has no efficacy against FHV-1 in vitro.[2]

Results of the present study provided data that advanced the understanding of the PK of penciclovir in tears, which is critical if famciclovir is to be used to treat herpetic disease that involves the ocular surface, especially the avascular cornea. In cats, concentrations of drugs in tears following oral administration vary and range from undetectable (doxycycline) to concentrations that exceed plasma concentrations (pradofloxacin).[28] For the cats of the present study, the penciclovir concentration in tears was approximately 20% of that in plasma. Simply modeling tear drug concentration as a fraction of the plasma drug concentration provided an acceptable fit for the observed data for penciclovir, but provided a poor fit for the BRL42359 data. Compared with the total tear volume, basal tear turnover is rapid,[29] and tears are derived by continuous replacement from plasma. Therefore, the proportionate flow-based model used in the present study is physiologically reasonable. However, it is unclear to what extent reflex tear production stimulated by the STT strips altered drug concentrations in tears.[30] Additionally, cats with herpetic disease will likely have abnormal tear dynamics associated with ocular discomfort, corneal surface disruption, conjunctival capillary fragility, and altered surface mucus, which might affect drug concentration in tears following famciclovir administration. For example, in a pilot study[21] conducted by our laboratory group, 7 cats with spontaneous ocular disease believed to be herpetic in origin had median tear penciclovir concentrations (197 to 1,095 ng/mL) that varied greatly following administration of famciclovir (40 mg/kg, PO, q 8 h) for at least 24 hours, although the median tear penciclovir concentration for all 7 cats in that study (455 ng/mL) was fairly similar to that for the healthy cats of the present study following administration of the 40 mg/kg-every-8-hours regimen (median, 217 ng/mL; mean, 300 ng/mL).

Although the PK data of the present study were novel and advanced the understanding of famciclovir metabolism in cats, those data were generated from a homogeneous population. Additional studies should be conducted with cats of varying breed, sex, and age and should include cats with liver or kidney dysfunction in particular. In human patients administered famciclovir, the rate of penciclovir clearance from plasma is reduced in patients with advanced age or kidney dysfunction,[25,31] and patients with liver disease have reduced BRL42359 metabolism.[25] Consequently, a decrease in famciclovir dosing frequency is recommended for humans with kidney dysfunction,[31] and the same may be necessary for cats with kidney disease. Even though famciclovir dosage is typically not altered in humans with liver dysfunction, it may need to be altered in cats with liver disease because cats inherently have limited hepatic aldehyde oxidase activity (an enzyme required for BRL42359 metabolism), and the activity of that enzyme may be further compromised in cats with liver disease.

One cat developed acute urethral obstruction during the present study. Although the underlying cause for the urethral obstruction in that cat was not determined, several things make us believe that the obstruction was not an adverse effect of famciclovir administration. The cat did not develop clinical signs of the obstruction until 16 days after administration of the last dose of famciclovir in phase 3. The serum creatinine concentration for that cat at the end of phase 3 (1 mg/dL) was the same as that prior to initiation of phase 1 and was just below the reference range (1.1 to 2.2 mg/dL). Finally, evidence in the scientific literature suggests that famciclovir is fairly safe for administration to human patients with kidney disease and cats with herpetic disease. For example, famciclovir is used as a rescue antiviral drug for human patients with renal toxicosis induced by administration of acyclovir.[32] Furthermore, in a study[9] in which 59 client-owned cats with suspected herpetic disease were treated with famciclovir, only 10

(17%) developed adverse effects potentially attributable to famciclovir; only 1 of those 10 cats had adverse effects referable to the urinary tract (polydipsia without a concurrent decrease in urine specific gravity). Adverse effects were not associated with the dosage of famciclovir (40 or 90 mg/kg, PO, q 8 h) administered in that study.[9] Therefore, we believe that the urethral obstruction developed by the cat in the present study was a random occurrence rather than an adverse effect associated with famciclovir administration. However, we advise prudence when famciclovir is administered to cats, especially those with impaired kidney function, and further studies are necessary to elucidate the safety of famciclovir in cats.

Our intent for conducting the present study was to generate data that could be used to better define an appropriate dose of famciclovir for cats infected with FHV-1. This was important because suboptimal concentrations of antiviral drugs are likely to promote selection of drug-resistant viral strains.[33] We evaluated 2 PK-PD indices ($T > IC_{50}$ and AUC_{24}:IC_{50}) that have been extensively used in antimicrobial research to facilitate determination of optimal dosages and thereby improve antimicrobial efficacy and reduce the emergence of resistance.[34] The C_{max}:IC_{50} was not evaluated in the present study because the C_{max} varied greatly among cats, which might have affected interpretation of that index. Given that penciclovir inhibits viral DNA polymerase and assuming simple Michaelis-Menten enzyme kinetics with no other PD effects, a $T > IC_{50}$ model of drug action was likely most appropriate for the present study.[35,36] Interpretation of that index requires determination of the optimal $T > IC_{50}$. Generally, a $T > IC_{50}$ that ranges from 40% to 50% of the dosing interval is considered necessary to achieve a cure by the use of time-dependent antimicrobials such as penicillins[37]; however, to our knowledge, similar data for virostatic drugs such as penciclovir are currently unavailable.

In the present study, when the lowest reported IC_{50} (304 ng/mL) was used as the target concentration, all dosage regimens evaluated achieved a $T > IC_{50}$ of > 94%. When 860 ng/mL (the IC_{50} previously calculated for the same viral isolate in the only prospective, placebo-controlled famciclovir efficacy trial[6] to date) was used as the target concentration, a $T > IC_{50}$ of > 50% was achieved in plasma for all three 8-hour regimens and the 90 mg/kg-every-12-hours regimen; the $T > IC_{50}$ was < 50% for the other two 12-hour regimens evaluated. The $T > IC_{50}$ and AUC_{24}:IC_{50} for both plasma and tears achieved by the 90 mg/kg regimens were significantly greater than those for the 30 mg/kg and 40 mg/kg regimens. Collectively, the findings of the present study suggested that, in cats, the preferred dose of famciclovir is 90 mg/kg. This was consistent with results of another study[6] in which administration of famciclovir (90 mg/kg, PO, q 8 h) was an effective treatment for FHV-1 in experimentally infected cats. Likewise, in another study[9] that involved client-owned cats with suspected spontaneous herpetic disease, oral administration of famciclovir at a dosage of 90 mg/kg every 8 hours was more effective than administration of a dosage of 40 mg/kg every 8 hours. Administration of famciclovir at a dose of 90 mg/kg is especially important when the treatment target is an avascular tissue such as the superficial cornea where the drug reaches the tissue via the tear compartment but is also valid when the treatment target is vascularized tissues such as those affected by herpetic blepharitis, conjunctivitis, and vascularized stromal keratitis. On the basis of the results of the present study, the treatment efficacy of famciclovir orally administered at 90 mg/kg every 12 hours was predicted to be equivalent to that achieved with 90 mg/kg every 8 hours because both regimens achieved a $T > IC_{50}$ of > 50% in plasma and neither plasma or tear penciclovir exposure nor tear $T > IC_{50}$ varied significantly between those 2 regimens. However, actual tissue concentrations of penciclovir were not measured in the present study, and the results of the in vitro studies[2,3] from which the 2 IC_{50}s were selected for use in this study might not adequately reflect the complex and dynamic conditions present in vivo. Recommendation of a definitive dosage of famciclovir for the treatment of herpetic disease in cats will require in vivo comparison of the safety and efficacy of various dosages in cats experimentally or spontaneously infected with FHV-1.

The PK of famciclovir following oral administration to cats is complex and characterized by variable absorption and saturable metabolism. The rate-limiting step in the famciclovir metabolic pathway appears to be the conversion of BRL42359 to penciclovir, which is likely associated with the inherently low activity of hepatic aldehyde oxidase (the primary enzyme responsible for oxidation of BRL42359 to penciclovir) in domestic cats. On the basis of the observed penciclovir concentrations achieved in both plasma and tears and the calculated PK-PD indices of the present study, the recommended dosage regimen for famciclovir in cats is 90 mg/kg, PO, every 12 hours. Some veterinarians may elect to treat cats with famciclovir at a dose of 30 or 40 mg/kg to avoid having to partition tablets or to reduce costs for clients. However, the findings of this study do not support such a practice because oral administration of famciclovir at those doses (even when the drug was administered every 8 hours) failed to achieve adequate penciclovir concentrations in tears for therapeutic purposes and may be associated with the development of drug resistance.

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Footnotes

a. Famvir, Novartis Pharmaceuticals Corp, East Hanover, NJ.
b. Arrow International Inc, Reading, Penn.
c. Haag-Streit UK Ltd, Harlow, Essex, England.
d. Novartis Animal Health, Ringaskiddy, County Cork, Ireland.
e. AK Scientific Inc, Mountain View, Calif.
f. Sigma-Aldrich Corp, St Louis, Mo.
g. Phoenix WinNonLin, version 6.1, Pharsight Corp, Mountain View, Calif.
h. The Mathworks, Inc, Natick, Mass.
i. Stata/IC, version 13.1, StataCorp LP, College Station, Tex.

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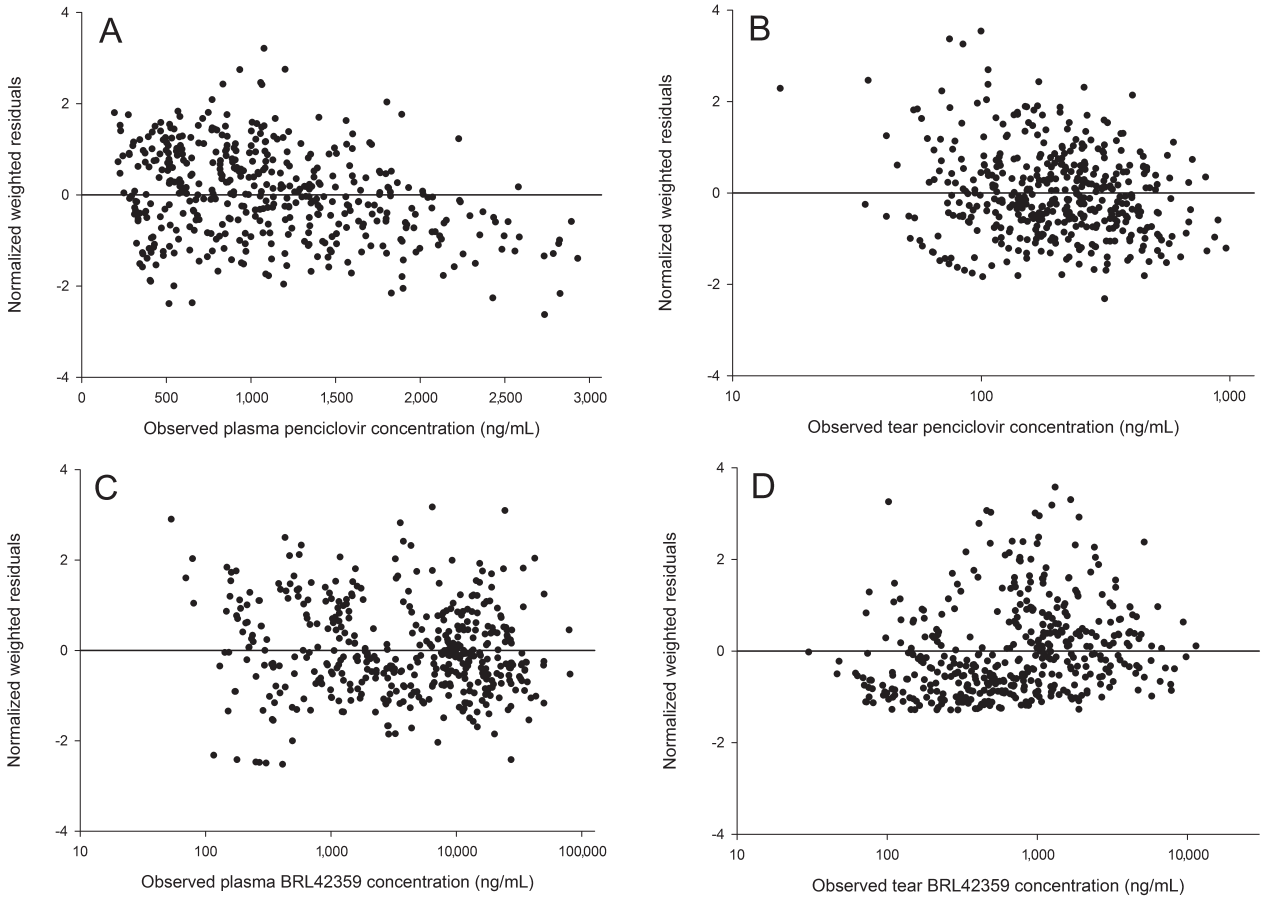
Appendices appear on next page

Appendix 1

Interday accuracy (as a percentage nominal concentration) and precision (as a percentage of the relative SD) of a liquid chromatography-tandem mass spectrometry assay for famciclovir, penciclovir, and BRL42359 concentrations in the plasma and tears of cats.

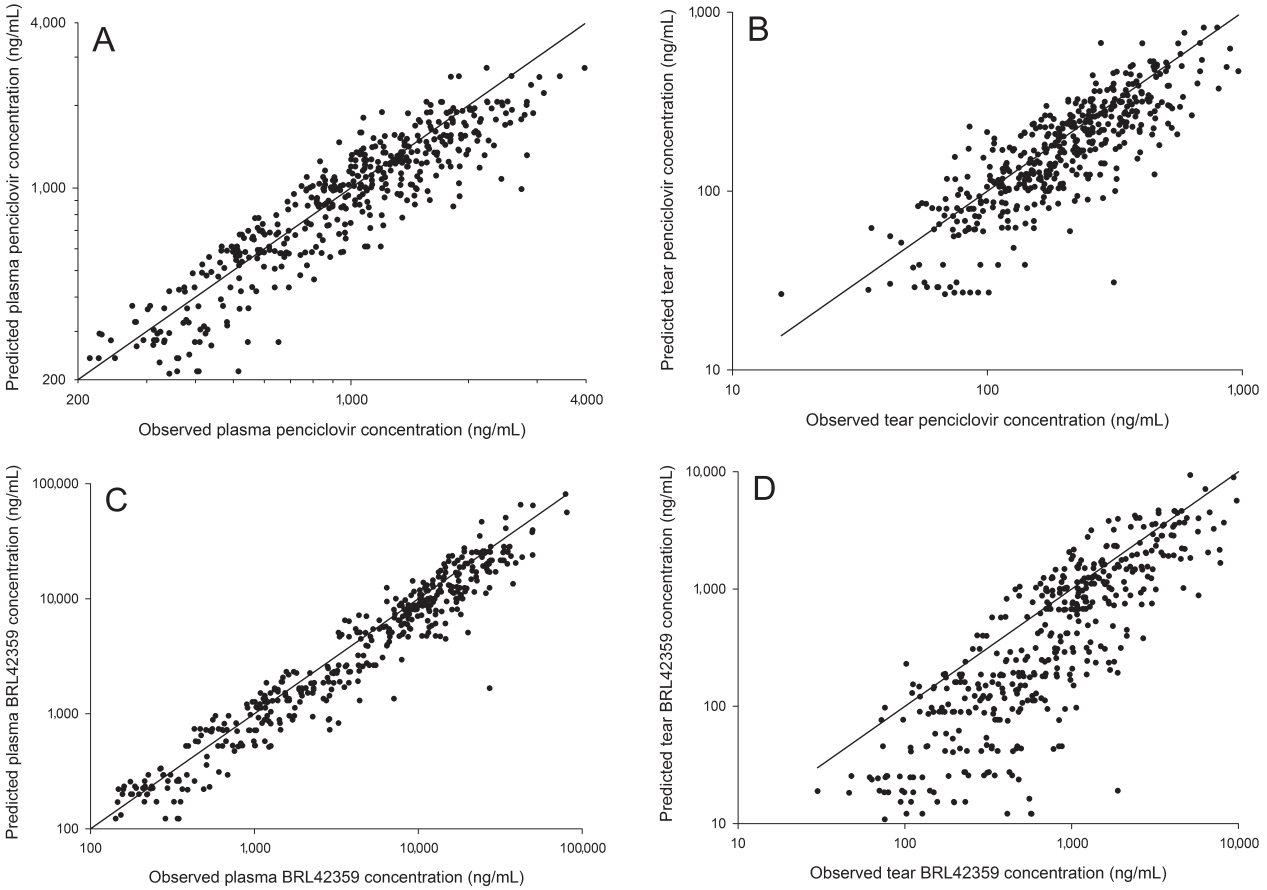
	Plasma			Tears		
Metabolite	Concentration (ng/mL)	Interday accuracy (%)	Interday precision (%)	Concentration (ng/mL)	Interday accuracy (%)	Interday precision (%)
Famciclovir	80.0	120	11.0	0.3	105	5.0
	800	112	5.0	10.0	104	6.0
	1,800	102	3.0	90.0	105	4.0
Penciclovir	6.0	107	6.0	—	—	—
	80.0	110	3.0	0.3	104	4.0
	800	105	4.0	10.0	102	6.0
	1,800	106	6.0	90.0	104	4.0
BRL42359	80.0	102	9.0	0.3	102	4.0
	800	95	8.0	10.0	103	5.0
	1,800	107	7.0	90.0	104	4.0
	18,000	100	5.0	—	—	—

— = Not determined.



Appendix 2

Scatterplots of the normalized weighted residuals versus the observed concentration of penciclovir (A and B) or BRL42359 (C and D) in the plasma (A and C) and tears (B and D) of 6 healthy sexually intact male domestic shorthair cats following oral administration of famciclovir at each of 3 doses (30, 40, and 90 mg/kg) for each of 2 dosing intervals (every 8 hours and every 12 hours) for 3 consecutive days. The study had a crossover design with 6 phases. During each phase, 6 cats were randomly assigned to a dosage regimen. There was a washout period of 4 days between each phase, except phases 3 and 4 when a 2-week rest period was observed. One cat developed acute urethral obstruction during the rest period between phases 3 and 4 and was replaced with another cat for the remainder of the study. Thus, 5 cats received all 6 dosage regimens and 2 cats each received 3 dosage regimens.



Appendix 3

Scatterplots of the predicted versus observed concentration of penciclovir (A and B) or BRL42359 (C and D) in the plasma (A and C) and tears (B and D) of the cats of Appendix 2. The solid line represents identity (ie, y = x). ***See*** Appendix 2 for remainder of key.