Veterinary Ophthalmology (2012) 1–8 DOI:10.1111/j.1463-5224.2011.00984.x

Pharmacokinetics of famciclovir and penciclovir in tears following oral administration of famciclovir to cats: a pilot study

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Abstract

Objective To validate a means of collecting tears from cats, develop an assay for quantifying famciclovir and penciclovir in tears, and to assess famciclovir and penciclovir concentrations and pharmacokinetics in the tears of cats being treated orally with famciclovir for suspected herpetic disease.

Animals Seven client-owned cats.

Procedures Cats were treated orally with a median (range) dose of 40 (39–72) mg of famciclovir/kg three times daily for at least 24 h. At various time points following famciclovir administration, tear samples were collected using Schirmer tear test strips. Tear famciclovir and penciclovir concentrations were measured using liquid chromatography-mass spectrometry, and concentration-time profiles were analyzed noncompartmentally. The relationship between famciclovir dose and tear penciclovir concentration near its maximum was evaluated using least squares linear regression.

Results Maximum tear famciclovir concentration of 0.305 μg/mL occurred at 2.64 h; elimination half-life was 2.28 h. Maximum tear penciclovir concentration (0.981 μg/mL) occurred 2.25 h following oral administration of famciclovir; elimination half-life was 2.77 h. A significant positive correlation was noted between famciclovir dose and tear penciclovir concentration at various time points between 0.5 and 3.75 h following drug administration ($P = 0.025$). Tear penciclovir concentration exceeded the concentration shown to have *in vitro* efficacy against feline herpesvirus (FHV-1) (0.304 μg/mL) in about half of samples collected.

Conclusions Oral administration of 40 mg of famciclovir/kg to cats resulted in a tear penciclovir concentration-time profile that approximated the plasma penciclovir concentration-time profile and frequently achieved a penciclovir concentration at the ocular surface likely to be effective against FHV-1.

Key Words: antiviral drugs, feline, feline herpesvirus-1, FHV-1, herpesvirus, pharmacology, tear film

INTRODUCTION

Penciclovir is a nucleoside deoxyguanosine analog with a mechanism of action similar to that of acyclovir and ganciclovir. It was developed for the treatment of disease caused by human herpesviruses, but also exerts potent *in vitro* antiviral action against feline herpesvirus (FHV-1),[1–5] and notably reduces clinical evidence of herpetic disease in cats.[6,7] However, penciclovir is poorly absorbed following oral administration in humans. Therefore, famciclovir, an oral prodrug of penciclovir, was formulated to enhance penciclovir bioavailability. Following gastrointestinal absorption in humans, famciclovir is metabolized to the active metabolite, penciclovir, by di-deacetylation and oxidation. The pharmacokinetics of penciclovir and famciclovir in cats appear to be very complex and to differ markedly in comparison with other species such as humans, dogs, and rats.[8–11] For example, famciclovir appears to be very poorly bioavailable in cats relative to humans. Bioavailability in humans administered a single oral dose of approximately 7 mg of famciclovir/kg is 77%[12] whereas in cats it is only 12.5% or 7% following oral administration of 40 or 90 mg of famciclovir/kg,

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respectively.[8] Once absorbed, the maximum plasma penciclovir concentration (C_{max}) achieved in cats administered a single dose of 90 mg of famciclovir/kg is 1.3 μg/mL, which is approximately one-third of that in dogs administered a single dose of only 25 mg of famciclovir/kg (4.4 μg/mL).[8,10] Furthermore, comparison of data from studies utilizing famciclovir at different dose rates in cats provides evidence of nonlinear pharmacokinetics in this species. A 2.7-fold increase in famciclovir dose (from 15 to 40 mg/kg) resulted in a fourfold increase in penciclovir C_{max} in cats; no further increase in C_{max} was observed when the dose was increased from 40 to 90 mg/kg.[8,9] Taken together, these data suggest that famciclovir is variably absorbed in cats,[8] that its metabolism to penciclovir becomes saturated at increasing doses,[8] and that the highest attainable plasma penciclovir C_{max} in cats is lower than in other species.[8–11]

Despite the low plasma penciclovir C_{max} attained in cats, orally administered famciclovir appears to be a particularly efficacious treatment for cats infected with FHV-1.[6,7] Two case series describing client-owned cats receiving a range of famciclovir doses demonstrated that treatment improves keratitis, conjunctivitis, dermatitis, or rhinosinusitis attributed to FHV-1.[6,7] Likewise, cats experimentally inoculated with FHV-1 and treated with 90 mg of famciclovir/kg orally three times daily (TID) showed significant improvement in numerous systemic, ophthalmic, clinicopathologic, virologic, and histologic parameters in comparison with results for these parameters in placebo-treated cats.[13] However, in this experimental study, there was no significant difference in the prevalence or duration of corneal ulcers observed in placebo- and famciclovir-treated cats.[13] This observation may reflect the inability of a systemically administered medication to achieve therapeutic concentrations at the corneal surface or within the avascular corneal stroma. Although orally administered valacyclovir or famciclovir has been used to treat epithelial keratitis caused by HSV-1 in humans and experimentally-induced HSV-1 infections in rabbits and mice,[14–16] to the authors' knowledge the concentration of penciclovir required at the corneal surface for effective control of herpetic disease has not been reported in any species, including humans.

Therefore, the purposes of this pilot study were to validate a means of collecting tears from cats *in vivo*, to develop an assay for quantifying famciclovir and penciclovir in tears, and to assess famciclovir and penciclovir concentrations and pharmacokinetics in the tears of cats being treated with orally administered famciclovir for suspected herpetic disease. This study also assessed whether the tear penciclovir concentrations achieved at this dose are potentially efficacious against FHV-1 using 0.3 μg/mL as the target penciclovir concentration. This was chosen because it is the lowest reported IC_{50} (concentration that inhibits viral growth *in vitro* by 50% relative to control wells) for penciclovir.[3]

MATERIALS AND METHODS

Animals and drug administration

All cats included in this study were client-owned cats presented to the Veterinary Ophthalmology Service of the University of California, Davis. Prior to inclusion, all cats received a complete ophthalmic examination by a veterinary ophthalmologist or resident in training and were diagnosed with clinical signs consistent with herpetic disease. All cats were administered oral doses of famciclovir TID for at least 24 h prior to sample collection to ensure that steady-state plasma concentrations were achieved.[8,13] All cats received famciclovir as whole (n = 3) or halved (n = 4) tablets from Novartis (n = 2), TEVA pharmaceuticals (n = 2), or an unknown manufacturer (n = 3). This study was approved by the Institutional Clinical Trial Review Board (Proposal # 10-02-40), and all owners gave informed consent to their cats being included in this study.

Sample collection and analysis

Tear samples for assessment of famciclovir and penciclovir concentration were collected using Schirmer tear test (STT) strips. Prior to use, each STT strip was placed in an individual cryovial and a baseline mass of the dry strip within its cryovial was attained. Each STT strip was then placed in the ventrolateral conjunctival fornix of one eye, left for approximately 1 min, 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 assuming that 1 g and 1 mL of tears were equivalent. Following weighing, each STT strip was suspended in 2 mL of methanol and stored at −20 °C until quantification of penciclovir and famciclovir by liquid chromatography-mass spectrometry (LC-MS). Thirty-five tear samples were collected from the seven cats at 18 time points; samples were collected bilaterally on 17 occasions or unilaterally (OS only) on one occasion. The number of tear samples collected and the timing of sample collection relative to the most recent famciclovir administration and famciclovir therapy duration were determined by timing and frequency of the recheck examinations, which were determined by the clinician responsible for the care of the cat's ophthalmic condition. To permit comparison of tear drug concentration among cats with different degrees of conjunctival inflammation, severity of conjunctivitis was scored in six of the seven cats. Scoring of chemosis and conjunctival hyperemia was performed for each eye at each time point by the clinician responsible for the cat's care using a score of 0 (none), 1 (mild), 2 (moderate), or 3 (severe).

Liquid chromatography-mass spectrometry assay

For analysis of famciclovir (AK Scientific, Union City, CA, USA) and penciclovir (AK Scientific) in tear extracts, 200 ng of the internal standard acyclovir was added to each vial. After brief mixing, 1 mL of the tear extract was transferred to autosampler vials and dried under nitrogen. Samples were

then redissolved by adding 120 μL of 5% acetonitrile in water, with 0.2% formic acid, and briefly vortexed. Quantitative analysis was conducted on a Quantum Ultra triple quadrupole mass spectrometer (Thermo Scientific, San Jose, CA, USA). The high performance liquid chromatography system, column, and mobile phases were the same as those described above. The liquid chromatography pump provided a gradient of solvent A from 5.0% to 99% during 5.7 min at a flow rate of 0.6 mL/min. The injection volume was 40 μL. Detection and quantification employed selective reaction monitoring of initial precursor ion for famciclovir (mass to charge ratio [*m/z*] = 322.2). The response for the major product ions for famciclovir (*m/z* = 136.0, 280.1, and 262.1) was plotted and peaks at the proper retention time were integrated using commercial software (Xcalibur software version 2.0, Thermo Scientific, San Jose, CA, USA). Detection and quantification employed transitions of the initial precursor ion for penciclovir (*m/z* = 254.13). The response for the major product ions for penciclovir (*m/z* = 152.1, 135.1, and 110.1) was plotted and peaks at the proper retention time were integrated using the same commercial software. The internal standard acyclovir (*m/z* = 226.1) product ion response was monitored (*m/z* = 152.0, 135.0, and 110.1). The same Quanbrowser software was used to generate calibration curves and quantify these analytes in all samples. Quadratic equation calibration curves were used as a best fit for the calibrators using nominal concentrations from 0.01 to 50 ng/mL. All curves gave correlation coefficients (r^2) of ≥ 0.99. The limit of quantification was 0.01 ng/mL. Reported values were converted to mg/mL of tears by multiplying by 2 (to account for the 2 mL of methanol added to the sample) and dividing by the mass of tears collected (assuming that 1 mL and 1 g of tears were equivalent). Thus, reported tear penciclovir or famciclovir concentrations sometimes were less than the assay limit of quantification (0.01 ng/mL).

Data analysis

Analysis of pharmacokinetic data was performed with a commercial software program (Phoenix WinNonlin, version 6.2; Pharsight, Palo Alto, CA, USA.) and mean tear penciclovir and famciclovir concentration-time data following oral administration were assessed via noncompartmental analysis.[17] The C_{max} and T_{max} were estimated from the data. Linear trapezoidal areas were used in calculating the AUC, and other pharmacokinetic parameters were determined by use of standard noncompartmental equations. Specifically, the k_{el} was calculated as the slope of the terminal phase of the plasma-concentration curve that included a minimum of three points and $t_{1/2(\lambda_z)}$ was calculated as $t_{1/2(\lambda_z)} = 0.693/k_{el}$. For most analyses, tear penciclovir and famciclovir concentrations from the right and left eyes were averaged for cats in which both left and right eyes were sampled at the same time point. Least squares linear regression was used to evaluate the relationships between famciclovir dose and tear famciclovir concentration and between famciclovir dose and tear penciclovir concentration at various time points. Least squares linear regression was also used to evaluate the relationship between the conjunctivitis severity score and tear penciclovir concentration for individual eyes. A Wilcoxon rank sum test was used to assess differences between tear famciclovir and penciclovir concentrations. A paired difference *t*-test was used to assess differences in tear penciclovir concentrations between eyes with a different conjunctivitis score at the same time point. Significance was set at $P < 0.05$ for all analyses.

RESULTS

A total of seven client-owned cats (two neutered males and five spayed females) met all inclusion criteria (Table 1). The majority (4/7) were domestic short- or long-haired cats. Their median (range) body weight and age were 3.8 (3.1–5.0) kg and 12 (0.5–16) years, respectively. The major clinical diagnoses included keratitis, keratoconjunctivitis, symblepharon, and sequestra. Most (4/7) cats had bilateral disease. Median (range) famciclovir dose administered was 40 (39–72) mg/kg. All cats received famciclovir three times daily, as close to every 8 h as owners were able to achieve. Each cat was sampled between one and six times (Table 1)

Table 1. Demographic data for patients included in the study

Cat no.	Breed	Gender	Age (years)	Major clinical diagnoses	Famciclovir dose (mg/kg)	Number of tear samples collected	Duration of famciclovir therapy prior to first tear sample (days)	Median tear [famciclovir] (μg/mL)	Median tear [penciclovir] (μg/mL)
1	DLH	FS	16	Keratoconjunctivitis OD	39	2	24	0.864	0.455
2	DSH	FS	0.7	Keratitis OU	39	6	2	0.078	0.215
3	DSH	FS	0.5	Symblepharon OU	40	4	38	0.055	0.425
4	DLH	FS	14	Keratitis OD	64	3	20	0.059	0.815
5	Himalayan	MC	14	Keratoconjunctivitis OU	40	1	73	0.091	1.095
6	Persian	FS	2	Keratitis and sequestra OU	66	1	21	>LOQ	0.197
7	Himalayan	MC	12	Keratitis OS	72	1	165	0.028	0.799

DLH = domestic longhair; DSH = domestic shorthair; FS = female spayed; MC = male castrated; OD = oculus dexter (right eye); OS = oculus sinister (left eye); OU = oculi uterque (both eyes); LOQ = limit of quantification.

for a total of 18 individual samples throughout the study period. Median (range) duration of famciclovir therapy prior to collection of the first tear sample was 24 (2–165) days. Median (range) time between most recent famciclovir dose and tear sampling was 3.0 (0.5–11) h.

The LC-MS calibration curves for famciclovir and penciclovir were linear in the ranges 0.01–50 ng/mL (r^2 = 0.9951 and 0.9987, respectively). Quality control samples from both concentrations (4 and 20 ng/mL) were combined to assess accuracy and precision for famciclovir (87.9% and 5.3%, respectively) and penciclovir (92.4% and 5.7%, respectively) quantification in feline tears. The relative standard deviation was 6.5% for the famciclovir assay and 7.2% for the penciclovir assay. Tear famciclovir concentrations for Cat 6 appeared erroneously elevated in both eyes (greater than the limit of quantification for the right eye and 6.798 μg/mL for the left eye) and so were not included in data analysis; penciclovir concentration data from these tear samples were included.

Tear famciclovir concentrations varied greatly among individual cats (range 0.009–1.628 μg/mL; Fig. 1). However, when time-since-most-recent-dose and concentration data were averaged (Fig. 2), tear famciclovir concentration tended to decrease with increasing time since famciclovir administration. From the mean tear famciclovir-concentration-versus-time profile (Fig. 2), famciclovir C_{max} was 0.305 μg/mL and occurred 2.64 h following oral administration of famciclovir. Famciclovir elimination half-life was 2.28 h (Table 2). A significant correlation between famciclovir dose and tear famciclovir concentration was not detected regardless of whether data for all time points (P = 0.62) or for just those time points within 2 h of tear penciclovir C_{max} (0.5–3.75 h; P = 0.53) were considered.

Tear penciclovir concentrations among individual cats (range 0.084–1.395 μg/mL) ranged less widely than did tear famciclovir concentrations (Fig. 3). When time-since-most-recent-dose and concentration data were averaged, tear penciclovir concentration tended to decrease with increasing time since famciclovir administration (Fig. 4). Considering all samples collected from all time points, mean ± SD tear penciclovir concentration (0.495 ± 0.385 μg/mL) was significantly greater than mean tear famciclovir concentration (0.202 ± 0.397 μg/mL; P = 0.043). From the mean tear penciclovir-concentration-versus-time profile (Fig. 4), penciclovir C_{max} was 0.981 μg/mL and occurred 2.25 h following oral administration of famciclovir. Penciclovir elimination half-life was 2.77 h (Table 2). Considering data generated from all time points, a significant correlation between famciclovir dose and tear penciclovir concentration was not detected (P = 0.84; data not shown). However, comparison of data only from samples gathered within 2 h of tear penciclovir C_{max} (0.5–3.75 h; Fig. 5) revealed a significant (P = 0.025) positive correlation between famciclovir dose and tear penciclovir concentration.

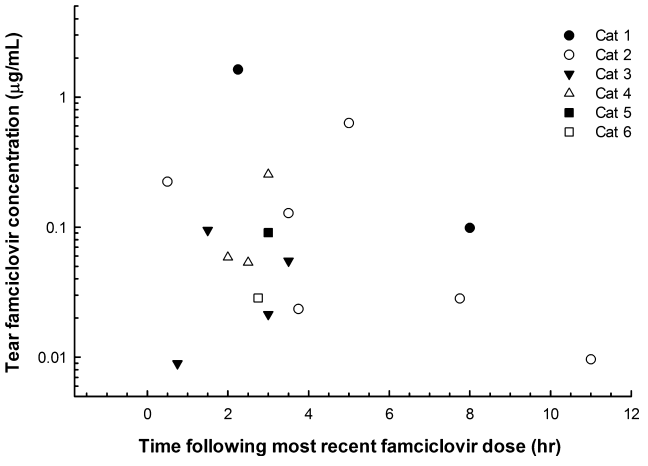

Figure 1. Observed tear famciclovir concentrations at various times following oral administration of 39–72 mg of famciclovir/kg three times daily to six cats.

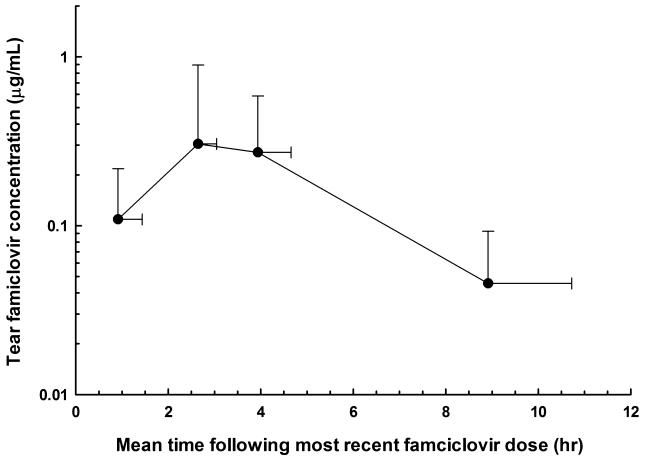

Figure 2. Averaged (mean + SD) data for tear famciclovir concentration vs. time following oral administration of 39–72 mg of famciclovir/kg three times daily to six cats.

Table 2. Tear pharmacokinetic values for famciclovir (n = 6 cats; 17 tear samples) and penciclovir (n = 7 cats; 18 tear samples) determined by noncompartmental analysis for mean concentration-versus-time profiles following multiple orally administered doses (39–72 mg/kg three times daily) of famciclovir to cats

Pharmacokinetic parameter	Famciclovir	Penciclovir
C_{max} (μg/mL)	0.305	0.981
T_{max} (h)	2.64	2.25
$C_{(avg)}$ (μg/mL)	0.171	0.395
$C_{ss(min)}$ (μg/mL)	0.109	0.284
$Fluctuation_{ss}$ (%)	114	177
AUC (μg h/mL)	1.42	3.30
$t_{1/2(\lambda_z)}$ (h)	2.28	2.77
Accumulation index	1.10	1.15

C_{max} = maximum plasma concentration; T_{max} = time to C_{max}; $C_{(avg)}$ = mean plasma penciclovir during the dosing interval at steady state; $C_{ss(min)}$ = minimum observed plasma penciclovir concentration during the dosing interval at steady state; $Fluctuation_{ss}$ = steady-state fluctuation; AUC = area under the plasma concentration–time curve during the dosing interval; $t_{1/2(\lambda_z)}$ = apparent elimination half-life.

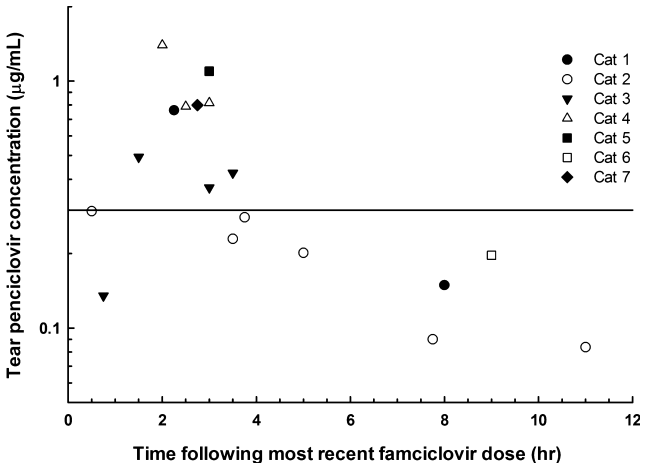

Figure 3. Observed tear penciclovir concentrations at various times following oral administration of 39–72 mg of famciclovir/kg three times daily to seven cats. The solid line represents the target penciclovir concentration of 0.3 μg/mL which is the lowest reported IC_{50} for penciclovir against FHV-1 *in vitro*.[3]

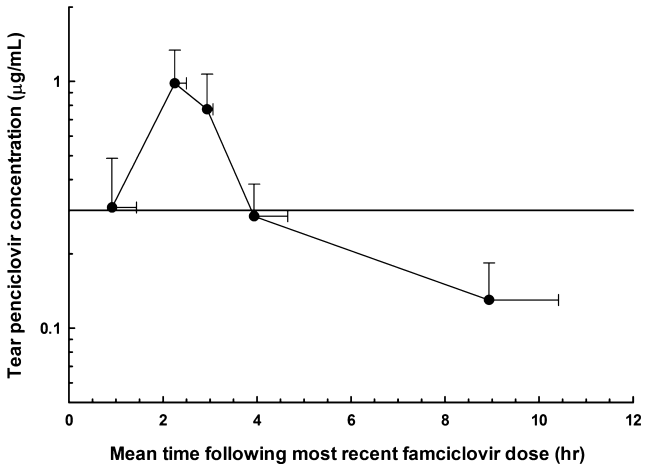

Figure 4. Averaged (mean + SD) data for tear penciclovir concentration vs. time following oral administration of 39–72 mg of famciclovir/kg three times daily to seven cats. The horizontal line represents the target penciclovir concentration of 0.3 μg/mL which is the lowest reported IC_{50} for penciclovir against FHV-1 *in vitro*.[3]

A significant correlation between severity of conjunctivitis and tear penciclovir concentration was not detected ($P = 0.78$) when all data were considered. However, there were five time points at which conjunctivitis scores differed between right and left eyes of the same cat. Considering just these occasions, the eye with the greater conjunctivitis score always had the greater tear penciclovir concentration. However, statistical comparison of this group of samples failed to reveal a significant difference ($P = 0.22$); eyes with greater conjunctivitis scores (median 1.5; range 1–2) had median (range) tear penciclovir concentrations of 0.823 (0.158–2.561) μg/mL while contralateral eyes with the lesser conjunctivitis score (median 0; range 0–1) had median (range) tear penciclovir concentrations of 0.229 (0.133–0.699) μg/mL.

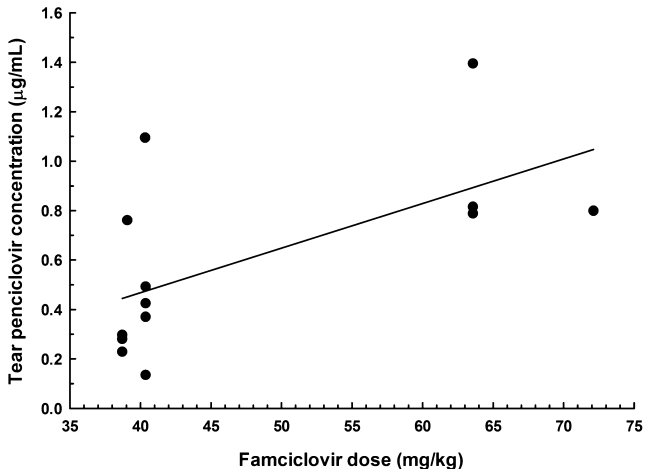

Figure 5. Correlation between famciclovir dose and tear penciclovir concentration in six cats sampled within 2 h of the maximum tear penciclovir concentration (0.5–3.75 h following oral administration of 39–72 mg of famciclovir/kg three times daily; $P = 0.025$). This period included the majority of samples in which tear penciclovir concentration exceeded the lowest reported IC_{50} for penciclovir against FHV-1 *in vitro* (0.3 μg/mL) were collected.[3]

Utilizing the lowest reported IC_{50} for penciclovir (0.3 μg/mL) as a target,[3] individual tear penciclovir concentration exceeded ($n = 9$) or was within one SD ($n = 2$) of this value in 11 of 18 samples (Fig. 3). Mean tear penciclovir concentration exceeded the target penciclovir concentration from approximately 1–4 h following famciclovir administration (Fig. 4).

DISCUSSION

To the authors' knowledge, this is the first study to evaluate the pharmacokinetics of famciclovir and penciclovir in the tears of any species. Using a clinically relevant population, this study demonstrated that oral administration of approximately 40 mg famciclovir/kg TID resulted in tear penciclovir concentrations likely to be efficacious against FHV-1 in a majority of cats. In addition, outcomes from this study confirmed that tear collection using STT strips was well tolerated, easily performed, and produced samples from which tear famciclovir and penciclovir concentrations could be assessed using novel LC-MS methodology with good accuracy and precision even at concentrations as low as 0.01 ng/mL. Previous studies have described use of STT strips[18–20] or microhematocrit tubes[21] for collection of tears for quantification of drug analytes in cats. Ideally, the technique for collection of tears for biochemical analysis should minimize the need for patient restraint, be well tolerated, and cause minimum stress and conjunctival irritation, thereby providing the best estimate of basal tear secretion and minimizing contamination of the tear sample with analytes from the blood. Although both microhematocrit tubes and STT would be expected to cause reflex tearing owing to contact with the conjunctiva or cornea, it seems likely that the risk

for conjunctival or corneal trauma would be greater with the microhematocrit tube, especially in patients with conjunctivitis or keratitis. In the present study, we did not demonstrate a correlation between severity of conjunctivitis and tear penciclovir concentration, which suggests that blood contamination of the tears with penciclovir or famciclovir was not excessive.

Comparison of data generated in this pilot study regarding the pharmacokinetics of famciclovir and penciclovir in tears with previous data for famciclovir and penciclovir pharmacokinetics in feline plasma permits formulation of some hypotheses regarding the means by which these two drugs enter feline tears. In particular, it is interesting to compare plasma pharmacokinetic data generated for cats that had received a single oral dose of 40 mg famciclovir/kg in a previous study[8] with tear pharmacokinetic data generated from cats in the current study following multiple doses of approximately 40 mg famciclovir/kg TID. Famciclovir C_{max} in tears in the present study (0.31 μg/mL) was approximately 10% of plasma famciclovir C_{max} (2.7 μg/mL), while famciclovir T_{max} in tears in the present study (2.5 h) was considerably longer than that calculated for plasma in the previous study (1 h).[8] However, famciclovir AUC, a measure of total drug exposure, was nearly identical for tears (1.42 μg h/mL) and plasma (1.32 μg h/mL)[8] in the two studies. Although data from these two studies must be compared with some caution because of differences in methodology (notably accuracy of dosing, number of doses prior to sample collection, and number and timing of samples collected), it appears that famciclovir is not concentrated in tears and appears there after some delay.

By contrast, penciclovir concentration-time profiles and pharmacokinetic parameters appear to be relatively similar in tears (following oral administration of approximately 40 mg of famciclovir/kg TID) and plasma (following a single oral dose of 40 mg of famciclovir/kg).[8] Penciclovir C_{max} achieved in tears (0.98 μg/mL) approximated that achieved in plasma (1.34 μg/mL),[8] and occurred at approximately the same time in tears (T_{max} = 2.3 h) as plasma (2.8 h). However, penciclovir AUC was less in tears (3.30 μg h/mL) than in plasma (13.1 μg h/mL) and half-life was shorter in tears (2.8 h) than in plasma (4.8 h).[8] Cautious comparison of these data sets, as they were generated under somewhat different conditions, reveals that penciclovir appears in and disappears from tears relatively rapidly, but that drug concentrations achieved in the two compartments are similar. Taken together, these data suggest that, following administration of approximately 40 mg famciclovir/kg TID to cats, penciclovir likely diffuses into the tears more readily than does famciclovir or that some active transport of penciclovir may occur. By contrast, active transport is likely not a predominant mechanism by which famciclovir reaches tears. More likely, famciclovir diffuses relatively slowly from plasma into the lacrimal gland or is released into tears relatively gradually from the lacrimal gland.

In addition to transport mechanisms, the concentration of antimicrobial compounds in tears is influenced by a number of intrinsic properties of the drugs (protein binding, lipophilicity, molecular weight, degree of ionization) and characteristics of the tears themselves (pH, flow rate, etc.).[18] Plasma protein binding exerts a particularly important influence on a compound's distribution in interstitial fluids. As a rule, more highly protein-bound compounds distribute less well into low-protein fluids such as tears than do less protein-bound compounds.[22] While protein binding for penciclovir has not been measured in the plasma of cats, plasma protein binding of penciclovir in humans, dogs, and rats is only approximately 20%.[10,23] If protein binding is similar in cats, this may help explain the relatively rapid and complete movement of penciclovir from plasma to tears in this species. To the authors' knowledge, protein binding for famciclovir has not been reported in any species. By contrast, highly lipophilic compounds diffuse readily across capillary walls and transcellularly.[18] Knowledge that famciclovir is more lipophilic than penciclovir owing to absence of the 6-deoxy group on famciclovir[24] may help explain why famciclovir persists in the tears longer than in plasma from where it is rapidly metabolized and/or excreted.[8] Molecular weights of famciclovir (321.14 g/mol) and penciclovir (253.25 g/mol) are relatively similar and likely play a minor role in the relative tear pharmacokinetics of the two drugs. Tear pharmacokinetics in cats have been reported for relatively few other drugs. Pradofloxacin has a shorter T_{max} and markedly greater C_{max} in tears than in plasma of cats, which has led to the assumption that active transport may play an important role in pradofloxacin secretion into tears.[18] By contrast, doxycycline did not reach detectable concentrations in feline tears suggesting that its high protein binding may inhibit its diffusion from the plasma into the tears, despite its relatively high lipophilicity.[18]

In the current study, a significant positive correlation between famciclovir dose and tear penciclovir concentration was noted when time points within 2 h of C_{max} (between 0.5 and 3.75 h following famciclovir administration) were considered. This time range was chosen because tear penciclovir concentrations decreased rapidly beyond 4 h (Fig. 4), which introduced additional variability into the data set. In addition, all tear samples that exceeded the target penciclovir concentration of 0.3 μg/mL were collected between 0.5 and 3.75 h following famciclovir administration. This correlation between famciclovir dose and tear penciclovir concentration contrasts with data from a previous study in which no difference in plasma penciclovir concentration was noted for cats receiving 40 or 90 mg of famciclovir/kg per os TID.[8] It is probably also important that mean tear penciclovir concentration in this current study exceeded the same target penciclovir concentration (0.3 μg/mL) for only approximately 3 h, from approximately 1–4 h following famciclovir administration. Although more thorough investigation of these initial observations is required, these data suggest that higher doses of famciclovir given at least three times daily

may be useful for treatment of keratitis in which corneal vascularization is not sufficient to guarantee drug delivery. However, determining an appropriate target penciclovir concentration likely to be effective in cats with spontaneously occurring herpetic disease remains challenging using the data currently available for a number of reasons. *In vitro* estimates of the IC_{50} for penciclovir against FHV-1 vary widely (0.3–33 μg/mL).[1–5] Using the lowest of these reported IC_{50}s (0.3 μg/mL),[3] 11 of 18 tear samples collected in the present study had penciclovir concentrations that exceeded or were within one SD of this target concentration. However, the relevance of *in vitro* IC_{50} data to target concentrations *in vivo* is not clear. For example, approximate peak plasma penciclovir concentrations of 2.0–2.1 μg/mL following 90 mg of famciclovir/kg per os TID were highly efficacious in decreasing clinical signs and viral shedding in experimentally infected cats.[13] However, in a separate study,[6] administration of as little as 8 mg famciclovir/kg once daily to client-owned cats reduced clinical signs of conjunctivitis, keratitis, rhinosinusitis, and dermatitis attributable to FHV-1 but not confirmed to be due to herpetic infection. Although plasma penciclovir concentrations were not reported for those cats,[6] previous pharmacokinetic data[9] suggest that the clinical efficacy noted was achieved at plasma penciclovir concentrations likely to be lower than those reported to be efficacious *in vivo*.[13] Tear penciclovir concentrations in the present study occasionally exceeded trough plasma penciclovir concentrations (0.5 μg/mL), but never exceeded peak plasma penciclovir concentrations (2.0 μg/mL) reported to be effective *in vivo*.[13] The correlation between famciclovir dose and tear penciclovir concentration shown here, and comparison of tear penciclovir data from the present study with previous *in vitro* and *in vivo* efficacy data, when assessed together, suggest that 40 mg famciclovir/kg administered TID in cats may be useful for treatment of ulcerative herpetic keratitis even in the absence of corneal vascularization. However, more information about penciclovir penetration into the corneal stroma and aqueous humor from the tears and across the blood aqueous barrier would be useful before judging the appropriateness of this therapy for herpetic stromal keratitis or anterior uveitis. To the authors' knowledge, penciclovir concentrations in cornea or aqueous humor have not been reported for other species receiving orally administered famciclovir. However, penciclovir concentration in the vitreous following oral administration of 500 mg famciclovir TID in humans undergoing elective pars plana vitrectomy was approximately one-third of the plasma concentration.[25] Despite this, the vitreous penciclovir concentration (1.2 μg/mL) was sufficient to be efficacious against human herpesviruses.

The present study has several limitations typical of small, prospective pilot studies utilizing client-owned patients. For example, administration of famciclovir and reporting of time of last dose was dependent completely on client compliance. Administration of famciclovir from different manufacturers and as whole and/or halved tablets with or without concurrent food administration also introduced variability into the present study. In addition, the age, breed, gender, and ocular signs of cats varied more in the present study than in typical experimental studies. Owing to the small population investigated and the paucity of tear samples collected at ideal times following drug administration in individual cats, population-based pharmacokinetic analysis was utilized in the present study, which did not allow for measurements of variability or statistical comparisons. Finally, it would have been ideal to obtain tear and blood samples simultaneously so that direct comparisons between the penciclovir concentrations in tears and plasma could have been performed. Nevertheless, this study utilized a clinically relevant population to demonstrate that oral administration of famciclovir at approximately 40 mg/kg TID resulted in penciclovir concentrations in the tears that are likely to be efficacious against FHV-1 in a majority of cats. In addition, outcomes from this study confirm that the tear collection methodology using STT strips was well tolerated, easily performed, and produced samples from which tear penciclovir concentrations could be assessed. This study also validated the novel HPLC methodology used for quantification of tear penciclovir concentration. Finally, this pilot study is, to the authors' knowledge, the first to evaluate the pharmacokinetics of famciclovir and penciclovir in the tears of any species. These data will inform future experimental and clinical studies evaluating the pharmacokinetics and efficacy of famciclovir and penciclovir in cats and other species.

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