Evaluation of orally administered famciclovir in cats experimentally infected with feline herpesvirus type-1

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Objective—To evaluate orally administered famciclovir for treatment of cats with experimentally induced disease attributable to feline herpesvirus type-1 (FHV-1).

Animals—16 nonvaccinated specific-pathogen–free cats.

Procedures—Cats were treated orally with famciclovir (90 mg/kg; n = 10) or a similar volume of lactose (400 mg; 6) 3 times/d for 21 days. Cats were inoculated with FHV-1 and administered the first treatment dose on day 0. Disease score; weight; results of urinalysis, serum biochemical analysis, and CBC; histologic conjunctivitis score; herpetic DNA shedding; goblet cell density; anti–FHV-1 antibody concentration; and plasma penciclovir concentration were measured.

Results—On days 4 to 18 following inoculation, disease scores were lower in famciclovir-treated cats than in lactose-treated cats. Lactose-treated cats decreased in weight during the first 7 days after inoculation, but famciclovir-treated cats increased in weight throughout the study. Percentage change in weight was greater in famciclovir-treated cats increased in weight throughout the study. Percentage change in weight was greater in famciclovir-treated cats on days 7 and 14 than in lactose-treated cats. Serum globulin concentration was lower on days 3 through 9, conjunctivitis histologic score was lower on day 14, herpetic DNA was shed less frequently throughout the study, goblet cell density was greater on day 21, and circulating anti–FHV-1 antibody concentration at study end was lower in famciclovir-treated cats, compared with these measurements in lactose-treated cats. Approximate peak plasma penciclovir concentration was 2.0 µg/mL.

Conclusions and Clinical Relevance—Famciclovir administration improved outcomes for systemic, ophthalmic, clinicopathologic, virologic, and histologic variables in cats experimentally infected with FHV-1. Adjunctive topical mucinomimetic and antimicrobial treatments may also be necessary. (Am J Vet Res 2011;72:85–95)

Abbreviations

C Maximal observed plasma concentration
FHV-1 Feline herpesvirus type-1
GCD Goblet cell density
HSV Herpes simplex virus
IC50 Half maximal inhibitory concentration
IQR Interquartile range
k Rate constant
qPCR Quantitative PCR
STT Schirmer tear test
TFBUT Tear film breakup time
Tlag Absorption lag time
Vdss/F Apparent volume of distribution at steady state divided by bioavailability

Feline herpesvirus type-1 is a common cause of respiratory and ocular disease in cats. Kittens infected with FHV-1 typically have moderate to severe ocular disease and disease of the nasal cavity, paranasal sinuses, pharynx, or larynx; in addition, death can occur.1 Following primary infection with FHV-1, lifelong neural latency is established in approximately 80% of cats, with periods of viral reactivation throughout the
remainder of life in many cats. Herpetic infection may be associated with conjunctivitis, keratitis, corneal sequestration, eosinophilic keratitis, anterior uveitis, rhinosinusitis, and dermatitis. Therefore, primary and recurrent disease caused by feline herpesvirus comprises a diverse range of common and sometimes chronic or recurrent clinical syndromes that may cause frustration for veterinarians who attempt to treat them.

Antiviral drugs approved for the treatment of cats infected with FHV-1 do not exist in the United States. However, several antiviral agents developed for the treatment of herpetic disease in humans have been used in cats. Unfortunately, many of these drugs have low efficacy for the treatment of FHV-1,12,13 have poor bioavailability in cats,16 or are toxic when administered systemically in cats. Toxicosis is of less concern when ophthalmically applied antiviral agents developed for use in humans are used to treat affected eyes of cats, but concerns regarding their efficacy against FHV-1 infection remain. Furthermore, most antiviral agents require frequent application.12,13 Therefore, discovery of an effective and safe systemic antiviral agent for the treatment of cats infected with FHV-1 is an important goal.

Recently, investigators have studied the anti–FHV-1 activity of penciclovir. Penciclovir is a nucleoside deoxyguanosine analog with a mechanism of action similar to that of acyclovir and with potent antiviral activity against the human herpesviruses (ie, HSV-1, HSV-2, and varicella zoster virus). The IC₅₀ of penciclovir was determined to be 13.9 µM (3.5 µg/mL) when used against FHV-1 in in vitro cultures.13 This is similar to that of idoxuridine and cidofovir, which are clinically useful when applied topically for the treatment of FHV-1 infection. In addition, penciclovir is more potent than acyclovir, which is the only other drug used systemically to treat cats infected with FHV-1.11–13,17 However, because of poor bioavailability of orally administered penciclovir, the prodrug famciclovir is prescribed for use in humans. Following absorption, famciclovir is metabolized to penciclovir by di-deacetylation and oxidation.18 The pharmacokinetics of penciclovir after the oral administration of famciclovir in healthy cats have been reported.19 Oral administration of 9 to 18 mg of famciclovir/kg every 8 or 12 hours in cats was not associated with toxic effects; however, the Cₚ₀ was 5- to 10-fold less than the target concentration (IC₅₀) of 3.5 µg of penciclovir/mL reported in another in vitro study.13 Analysis of data from a pharmacokinetic study19 suggested that the pharmacokinetics of famciclovir and penciclovir are complex, but computer modeling predicted that a dosage of 90 mg of famciclovir/kg administered orally 3 times daily would achieve plasma concentrations that approximated the reported IC₅₀ of penciclovir.13 Therefore, the purpose of the study reported here was to evaluate the efficacy and safety of 90 mg of famciclovir/kg when administered orally 3 times daily for the treatment of cats with experimentally induced disease attributable to primary FHV-1 infection, and to further elucidate the complex pharmacokinetics of penciclovir following administration of its prodrug famciclovir.

Materials and Methods

Animals—Ten sexually intact male and 6 sexually intact female nonvaccinated specific-pathogen–free domestic shorthair cats were included in the study. The mean ± SD body weight and age were 3.3 ± 0.6 kg and 0.47 ± 0.04 years, respectively. These 16 cats had no known history of ocular or systemic illness. Prior to inclusion in this study, all cats were screened and determined to be free of anti-FIV19 and anti–FHV-110 antibodies and FelV antigen. Cats were assessed as healthy on the basis of results of physical examinations, CBCs, serum biochemical analyses, and urinalyses performed prior to the start of the study (baseline). The ambient temperature (ie, 21°C ± 2°C) and light-to-dark cycle ratio (ie, 14 hours of light to 10 hours of darkness) of the housing area were controlled. All cats were housed separately in the same room and had ad libitum access to fresh water and a commercially prepared dry expanded diet. The proximate dry-matter composition of the diet was 33.4% crude protein, 16.3% crude fat, 7.2% ash, and 1.3% crude fiber. The study was approved by the Institutional Animal Care and Use Committee of the University of California-Davis.

Study design—Cats were assigned to treatment groups by assigning the first 10 cat identification numbers drawn from a hat to the famciclovir treatment group and the remaining cats to the lactose treatment group. Cats were allowed a 48-hour acclimatization period. Then, 10 cats (6 males and 4 females) received famciclovir19 (90 mg/kg, PO, 3 times daily [8:00 AM, 2:00 PM, and 8:00 PM] for 21 days, and 6 cats (4 males and 2 females) received a similar volume of lactose (400 mg, PO, 3 times daily [8:00 AM, 2:00 PM, and 8:00 PM]) for 21 days. Both treatments were prepared in gelatin capsules for ease of administration. Cats were restrained while capsules were administered by use of a plastic pill-administration device, which was followed immediately by the administration of approximately 5 mL of water via a syringe. A single pill-administration device was used on all cats but was cleaned with water between each pill administration to ensure that the gelatin capsules did not inadvertently receive trace amounts of famciclovir that may have leaked from within the gelatin capsules. Each cat was individually assigned a syringe for water administration. All cats were monitored for approximately 5 minutes after treatment administration to ensure that the gelatin capsules were swallowed. All cats were administered the initial dose of famciclovir or lactose and immediately afterwards inoculated with passage 8 of a strain of FHV-1 that is a plaque-purified field isolate verified as FHV-1 by use of PCR assays because contamination with Chlamydophila felis, or feline calicivirus by use of PCR assays because contamination with those organisms may have confounded the clinical disease induced in the inoculated cats. The viral inoculum comprised approximately 3.2 × 10⁷ plaque-forming units of the 727 strain of FHV-1 and was topically administered in approximately equal portions into both nares and both conjunctival fornices. This same dose
of virus has been used in other studies\textsuperscript{20,21} and reliably induces clinically overt but self-limiting disease in specific-pathogen–free cats.

**Clinical assessment**—Before inoculation and for 21 days after inoculation with FHV-1, cats underwent physical examinations twice daily (8:00 AM and 8:00 PM); examinations included assessments of rectal temperature, pulse and respiratory rates, hydration, mucous membrane color, and behavior and also auscultation of the thoracic cavity. Prior to inoculation with FHV-1 and for 21 days thereafter, clinical signs of disease (ie, ocular discharge, blepharospasm, and conjunctivitis) caused by FHV-1 infection were assessed for each eye once daily in accordance with a previously reported\textsuperscript{21} scoring system. Disease scoring was conducted by 1 of 2 trained evaluators (DJM or CCL) who were unaware of treatment assignments. Severity of ocular discharge was assigned a score of 0 (none) through 3 (marked mucopurulent discharge). Severity of blepharospasm was assigned a score of 0 (none) through 4 (eye completely closed). Severity of conjunctivitis was assigned a score of 0 (none) through 3 (severe). In addition, sneezing (score = 0 [absent] or 1 [present]) and severity of nasal discharge (score = 0 [none] through 3 [marked mucopurulent discharge]) were assessed. Total clinical disease score was defined as the sum of all ocular (conjunctivitis, blepharospasm, and ocular discharge) and nonocular (sneezing and nasal discharge) scores. The minimum and maximum total clinical scores possible were 0 and 24, respectively.

Before inoculation and on days 3, 6, 9, 12, 15, and 19 after inoculation, each cat also received a complete ophthalmic examination that included assessment of pupillary light and palpebral reflexes and menace response, slit-lamp biomicroscopy of the anterior segment, STT values and TFBUT measurements, and fluorescein staining of the cornea (in that order). Aqueous tear production, which was assessed by placement of a standardized STT strip and measurement of the number of millimeters that became wet in 1 minute, was performed with strips from the same lot number.\textsuperscript{22} Tear film stability was assessed by measuring TFBUT as described elsewhere.\textsuperscript{23} To ensure accurate assessment of STT values and TFBUT measurements, only 1 investigator (CCL) performed these tests and a stopwatch was always used to measure the elapsed time. Personnel performing the STT or TFBUT assessments (CCL) and ophthalmic examinations (CCL or DJM) were unaware of the group assignment for each cat. Blood was collected from the jugular or medial saphenous vein of each cat for CBC and serum biochemical analysis before inoculation and on days 3, 6, 8 or 9; 12; 15; and 19, 20, or 21 after inoculation.

**Histologic assessment**—Immediately before FHV-1 inoculation and on days 7, 14, and 21 after inoculation, conjunctival samples were obtained. After clinical assessments, a 0.5% proparacaine hydrochloride ophthalmic solution and 2% lidocaine hydrochloride gel were topically applied to the conjunctival surface, and a biopsy specimen of the conjunctiva was obtained from the ventromedial conjunctival fornix of the eye of each cat (biopsy of the conjunctiva alternated between the left and right eye for each collection period). Biopsy of the conjunctiva provided approximately a 3-mm\textsuperscript{3} tissue specimen. Conjunctival samples were placed in tissue cassettes with the epithelial surfaces oriented upward and then placed in neutral-buffered 10% formalin for histologic evaluation. Formalin-fixed conjunctival tissues were embedded in paraffin, sectioned, and stained with H&E or periodic acid–Schiff stain prior to evaluation via light microscopy.

All H&E-stained sections were examined by 1 investigator (CMR) who was unaware of group assignments. Sections were evaluated for the severity, type, and location of inflammation. Severity of inflammation was scored in accordance with a previously reported\textsuperscript{21} scoring system. Inflammation was assigned a score of 0 when inflammatory cells were absent or there were sparsely scattered individual inflammatory cells; it was considered normal when 1 or 2 subepithelial lymphoid follicles were observed without inflammation.\textsuperscript{24} Inflammation was assigned a score of 1 when scattered aggregates or diffusely distributed low numbers of inflammatory cells were observed. Inflammation was assigned a score of 2 when locally large or diffusely moderate numbers of inflammatory cells with or without mild distortion of tissue architecture were observed. Inflammation was assigned a score of 3 when diffuse infiltration or effacement of the mucosa by large numbers of inflammatory cells with distortion of tissue architecture was observed. Inflammation was further categorized on the basis of the predominant cell type or types observed (ie, neutrophilic, lymphoplasmacytic, or mixed) and predominant location of the inflammation (ie, epithelial, submucosal, or mixed). Tissues stained with periodic acid–Schiff stain were used to enumerate goblet cells. For each tissue stained with periodic acid–Schiff stain, 30 consecutive epithelial cells were counted, and the goblet cell-to-epithelial cell ratio (ie, GCD) was recorded by a single investigator (CCL) who was unaware of group assignments.

**Virologic assessment**—To verify that FHV-1 infection had developed in all cats, serologic testing for FHV-1\textsuperscript{1} was repeated on day 19 (n = 3 cats), 20 (5), or 21 (8) after inoculation. In addition, samples for quantification of FHV-1 and feline genomic DNA and RNA were collected from the inferior conjunctival sac before inoculation (day 0) and on days 1 through 7, 9, 11, 15, 17, 19, and 21 after inoculation. A 0.5% proparacaine hydrochloride ophthalmic solution was applied topically to each eye, and a single cytology brush\textsuperscript{1} was used to collect a sample by rolling the brush along the inferior conjunctival sac of both eyes. Care was taken so that the cytology brush did not touch any part of the eye other than the conjunctiva, and a single bottle of proparacaine was assigned for use in each cat throughout the study to avoid contamination of the sample with viral particles or DNA. Each cytology brush was immediately placed into a 1.5-mL microcentrifuge tube with 0.5 mL of a 1:1 solution of filtered PBS solution and nucleic acid purification lysis buffer\textsuperscript{1} and stored...
at 4°C until samples were processed for analysis via a qPCR assay.

In preparation for analysis by use of the qPCR assay, a lysate was prepared by vortexing each sample for 10 seconds. Then, the cytology brush was removed, proteinase K and 2 grinding beads were added, and the samples were homogenized by use of a homogenizer for 2 minutes at 1,000 strokes/min. Samples were stored at −20°C for ≥1 hour prior to incubation at 56°C for 30 minutes. After DNase digestion by use of a previously described technique, RNA was extracted from a 200-µL aliquot of the lysate by use of a semi-automated nucleic acid workstation. To confirm that all genomic DNA had been digested, a 1-µL aliquot from the extracted RNA sample was analyzed by use of a real-time qPCR assay that detected template DNA from the viral glycoprotein B L of a master mix containing 7.5, 37.5, and 75 nM of primer and genomic DNA, and 7 µL of a master mix containing proprietary primers and probes designed by use of a commercial software program. The PCR amplification conditions were an initial 2-minute duration at 50°C and a 10-minute duration at 95°C, followed by 40 cycles at 95°C for 15 seconds and at 60°C for 60 seconds by use of a real-time PCR system. Fluorescent signals were monitored and recorded during the annealing temperature phase of amplification, and cycle threshold values were recorded on the basis of a threshold of 0.04 and baseline values of 3 to 15. Each group of qPCR reactions included positive and negative control samples (purified DNA determined from FHV-1 and feline 18S gene). A second 200-µL aliquot of the original lysate was used to precipitate DNA by use of a kit, and the DNA was then extracted by use of the same semi-automated nucleic acid workstation.

All extracted samples were analyzed by personnel at another facility by use of a real-time qPCR assay that detected FHV-1 DNA from the viral glycoprotein B gene (GenBank accession No. S66371.1). To determine nucleic acid extraction efficiency, all samples were also analyzed for the presence of DNA of the feline 18S gene (GenBank accession No. X03205.1). Each qPCR reaction contained 5 µL of sample (diluted 1:5 for cDNA and genomic DNA) and 7 µL of a master mix containing proprietary primers and probes designed by use of a commercial software program. The PCR amplification conditions were an initial 2-minute duration at 50°C and a 10-minute duration at 95°C, followed by 40 cycles at 95°C for 15 seconds and at 60°C for 60 seconds by use of a real-time PCR system. Fluorescent signals were monitored and recorded during the annealing temperature phase of amplification, and cycle threshold values were recorded on the basis of a threshold of 0.04 and baseline values of 3 to 15. Each group of qPCR reactions included positive and negative control samples (purified DNA determined from FHV-1 and water, respectively). Viral load was calculated as previously described and was reported as the number of viral gene copies/10 copies of the feline 18S gene.

Plasma penciclovir concentration and pharmacokinetic analyses—Data from another pharmacokinetic study were analyzed by use of a commercial software program in an attempt to better predict the dosage and frequency of administration of famciclovir required to obtain a target penciclovir plasma concentration > 3.5 µg/mL for 4 to 8 hours daily. Data were simulated by use of a 1-compartment pharmacokinetic model with first-order absorption and elimination rates that was parameterized with Tlag (1.03 hours), Vd/F (11.53 L/kg), and an equal absorption and elimination k (0.35/h). Values for Tlag, Vd/F, and k were derived from mean pharmacokinetic parameters reported in cats orally administered multiple doses of 9 to 16 mg of famciclovir/kg every 8 hours. On the basis of simulations that used these parameters, a dosage of 90 mg of famciclovir/kg administered 3 times daily (8:00 AM, 2:00 PM, and 8:00 PM) was selected for the study. Blood samples were collected before inoculation with FHV-1 (day 0) and at times expected to approximate trough (8:00 AM and 2:00 PM) or peak (11:00 AM and 5:00 PM) plasma concentrations of penciclovir on days 1, 2, 4, 6, 8, 9, 12, 15, 19, and 20 or 21 after inoculation. All blood samples were collected by venipuncture from the jugular or medial saphenous vein into tubes containing sodium heparin and then were centrifuged at 2,000 X g for 10 minutes. Plasma was harvested from each tube and stored at −20°C for subsequent determination of plasma concentration of penciclovir via a liquid chromatography–mass spectrometry method described elsewhere. Pharmacokinetic analysis was performed by use of a commercial software program, and plasma penciclovir concentration-time data were assessed by use of noncompartmental analysis.

Statistical analysis—Age and body weight (2-tailed Student t test) and sex distribution (Fisher exact test) were compared between treatment groups for values recorded at baseline. For variables measured at multiple time points, a 2-way repeated-measures ANOVA was used to evaluate changes over time, differences in treatment groups, and time-by-treatment interactions. Normality of data was evaluated by use of a Shapiro-Wilk test. When data were not normally distributed, a logarithmic transformation was performed before the ANOVA or nonparametric tests were used to assess treatment-specific changes over time (Friedman test) or differences in treatments conditional on time (Mann-Whitney U test). Multiple comparison adjustments for post hoc comparisons were performed by use of a sequentially rejective adaptation of the Bonferroni method. The magnitude of the FHV-1 titer at the end of the study was compared between the famciclovir-treated and lactose-treated groups by use of a 2-tailed Student t test. The number of eyes with corneal ulcers and number of days for which corneal ulcers were observed, as well as the number of days for which FHV-1 DNA was detected in the conjunctival fornix, was compared between the famciclovir-treated and lactose-treated groups by use of a χ2 test of homogeneity. Normally distributed data were reported as mean ± SD, and data not normally distributed and ordinal data were reported as median ± IQR (ie, 25% to 75%). Statistical analyses were performed by use of software programs for repeated-measures ANOVA and nonparametric tests. A value of P < 0.05 was used to indicate significance for all analyses.

Results

Clinical assessment—At baseline, all cats were free of detectable disease (total clinical disease score = 0), and no significant differences were detected between cats in the famciclovir-treated and lactose-treated groups with respect to sex distribution (P = 1.00), age (P = 0.89), or body weight (P = 0.91). Following inoculation, all cats in both treatment groups developed clinical signs typical of primary FHV-1 infection, which included lethargy, fever, conjunctival hyperemia, chemosis, ocular discharge, sneezing, and nasal discharge.
Superficial or dendritic corneal ulcers were observed on day 9 and continued to be observed thereafter in all lactose-treated cats and 8 of 10 famciclovir-treated cats. No significant difference was detected between groups with regard to the number of eyes with corneal ulcers or the number of days on which corneal ulcers were observed (Figure 1). Median total clinical disease scores peaked on days 6 and 7 for the famciclovir-treated and lactose-treated cats, respectively (Figure 2). Clinical disease caused by FHV-1 infection was self-limiting, and 13 of 16 cats had a clinical disease score < 5 by day 20. Although all cats in both groups developed this typical pattern of disease caused by primary FHV-1 infection, median total disease score for famciclovir-treated cats was significantly less, compared with median total disease scores for lactose-treated cats, from days 4 through 18 (P = 0.003 to < 0.001 for each time point). No significant difference was detected between groups with respect to body temperature (P = 0.085) or heart rate (P = 0.64) throughout the study. However, respiratory rate on day 8 was significantly (P < 0.001) increased in the famciclovir-treated cats (64 ± 15 breaths/min), compared with the respiratory rate (35 ± 3 breaths/min) in the lactose-treated cats. Lactose-treated cats had a decrease in body weight during the first 7 days after FHV-1 inoculation. By contrast, famciclovir-treated cats had an increase in body weight for all measurements at each time point throughout the study (Figure 3). Mean percentage change in body weight was significantly greater in famciclovir-treated cats, compared with that in lactose-treated cats, on days 7 (5% and –13%, respectively; P = 0.004). Evidence of potential adverse effects after administration was not observed in any cat treated with famciclovir at any time throughout the study.

No significant difference was detected at baseline between mean STT values in cats treated with famciclovir (12 ± 6 mm/min) or lactose (8 ± 4 mm/min); however, the mean STT value for lactose-treated cats was below the reference range (11 to 23 mm/min) for cats. Mean STT values for both treatment groups remained within the reference range, and no significant (P = 0.54) differences in mean STT values were detected between treatment groups throughout the study (data not shown). Mean TFBUT of lactose-treated cats (22 ± 4 seconds) at baseline was above the reference range (12 to 21 seconds). In both treatment groups, mean TFBUT decreased during the study. By day 12, mean TFBUT for both treatment groups had decreased to less than the reference range and remained below the reference range for the remainder of the study (Figure 4).

No significant (P = 0.44) differences in mean TFBUT were detected between treatment groups throughout the study.
No remarkable abnormalities were detected at baseline for the results of CBCs, serum biochemical analyses, or urinalyses of any cat in the famciclovir-treated or lactose-treated groups. Serum globulin concentrations in famciclovir-treated and lactose-treated cats increased from baseline (3.75 ± 0.44 g/dL and 3.63 ± 0.16 g/dL, respectively), peaked approximately on day 6, and then returned to baseline concentrations on day 15. Mean serum globulin concentrations in famciclovir-treated cats on days 3 (4.01 ± 0.33 g/dL \( P = 0.005 \)), 6 (3.22 ± 0.24 g/dL \( P = 0.002 \)), and 8 or 9 (4.29 ± 0.33 g/dL \( P < 0.001 \)) were significantly lower, compared with mean concentrations in lactose-treated cats on days 3 (4.53 ± 0.26 g/dL), 6 (4.96 ± 0.30 g/dL), and 8 or 9 (4.58 ± 0.32 g/dL). However, serum globulin concentrations in cats from both treatment groups remained within the reference range (3.6 to 5.6 g/dL) on each of these days. Mean serum alkaline phosphatase activities in famciclovir-treated cats on days 6 (57 ± 26 U/L \( P = 0.01 \)), 8 or 9 (58 ± 24 U/L \( P = 0.005 \)), 12 (80 ± 18 U/L \( P < 0.001 \)), and 15 (89 ± 16 U/L \( P = 0.010 \)) were significantly higher, compared with mean activities in lactose-treated cats on days 6 (19 ± 6 U/L), 8 or 9 (23 ± 14 U/L), 12 (38 ± 12 U/L), and 15 (38 ± 23 U/L). However, mean serum alkaline phosphatase activities for famciclovir-treated and lactose-treated cats remained less than those determined at baseline (112 ± 23 U/L and 101 ± 19 U/L, respectively). Although some values for other variables measured with a CBC, serum biochemical analysis, or urinalysis were outside the reference ranges, no significant differences were detected.

![Figure 4](image1.png)
Figure 4—Mean + SD TF BUT in cats inoculated with FHV-1 (day 0) and then orally administered 90 mg of famciclovir/kg (n = 10 cats [black circles]) or 400 mg of lactose (6 [white circles]) 3 times/d (8:00 AM, 2:00 PM, and 8:00 PM) for 21 days. A reported reference range (12 to 21 seconds [dashed lines]) for TF BUT in cats is included for comparison. Mean TF BUT values did not differ significantly \( P = 0.44 \) between treatment groups throughout the study.

![Figure 5](image2.png)
Figure 5—Median ± IQR (ie, 25th to 75th percentile) histologic conjunctivitis score for cats inoculated with FHV-1 and then orally administered 90 mg of famciclovir/kg (n = 10 cats [black circles]) or 400 mg of lactose (6 [white circles]) 3 times/d (8:00 AM, 2:00 PM, and 8:00 PM) for 21 days. *On day 14, median histologic conjunctivitis score was significantly \( P = 0.005 \) greater in lactose-treated than in famciclovir-treated cats.

![Figure 6](image3.png)
Figure 6—Median ± IQR (ie, 25th to 75th percentile) GCD in cats inoculated with FHV-1 and then orally administered 90 mg of famciclovir/kg (n = 10 cats [black circles]) or 400 mg of lactose (6 [white circles]) 3 times/d (8:00 AM, 2:00 PM, and 8:00 PM) for 21 days. The GCD reference value is indicated (dashed line). *On day 21, median GCD was significantly \( P = 0.022 \) greater in famciclovir-treated than in lactose-treated cats. The GCD was the number of goblet cells/50 consecutive epithelial cells.

![Figure 7](image4.png)
Figure 7—Mean + SD FHV-1 DNA load in cats inoculated with FHV-1 and then orally administered 90 mg of famciclovir/kg (n = 10 cats [black circles]) or 400 mg of lactose (6 [white circles]) 3 times/d (8:00 AM, 2:00 PM, and 8:00 PM) for 21 days. Viral load was calculated as described elsewhere. *On day 4, FHV-1 viral DNA load was significantly \( P = 0.001 \) greater in lactose-treated than in famciclovir-treated cats.
between lactose-treated and famciclovir-treated cats on any day during the study. Changes in results of CBCs, serum biochemical analyses, or urinalyses that would be suggestive of drug toxicosis were not detected in any famciclovir-treated cats on any day.

**Histologic assessment**—Median histologic score for conjunctivitis at baseline was 1 in both treatment groups, and the inflammation consisted of a primarily lymphocytic population in the subepithelium. Following inoculation with FHV-1, increased histologic evidence of conjunctivitis was observed in all cats from both treatment groups, which peaked on day 7 and then returned to baseline in the famciclovir-treated cats on day 14 and in the lactose-treated cats on day 21 (Figure 5). On day 14, median histologic score (1; \( P = 0.005 \)) for conjunctivitis in famciclovir-treated cats was significantly lower, compared with the median histologic score (2) in lactose-treated cats. On day 7, inflammation of the conjunctiva in all cats was severe, included a mixed or neutrophilic population of inflammatory cells, and involved both the submucosa and epithelium. Ulcers of the mucosa were evident in all cats, except for 1 cat in the famciclovir-treated group. In cats with diffusely ulcerated mucosa, a neutrophilic exudate covering the conjunctival surface was observed. Intranuclear inclusion bodies were observed in 3 famciclovir-treated cats on day 7 and 1 lactose-treated cat on day 14. At the end of the study (day 21), all cats had mild persistent histologic evidence of conjunctivitis that was similar in magnitude to baseline but was characterized by a predominantly mixed or neutrophilic population of inflammatory cells and involved both the submucosa and epithelium. In both treatment groups, median GCD was marginally above the upper value of the reference range \(^{31} \) at baseline, decreased to almost 0 by day 7, and remained low for 21 days following inoculation with FHV-1 (Figure 6). At the end of the study, median GCD (4.5; \( P = 0.022 \)) was significantly greater in famciclovir-treated cats, compared with the median GCD (0) in lactose-treated cats.

**Virologic assessment**—All cats in both treatment groups seroconverted to FHV-1; however, at the end of the study, mean concentration of circulating anti–FHV-1 antibodies (1.2 ± 0.5 absorbance units; \( P = 0.001 \)) in famciclovir-treated cats was significantly less, compared with the mean concentration (2.8 ± 1.1 absorbance units) in lactose-treated cats. Before inoculation with FHV-1, no DNA or RNA of FHV-1 was detected via the qPCR assay in conjunctival cytobrush samples obtained from any cat. On day 2, DNA and RNA of FHV-1 were detected by use of the qPCR assay in all cats from both treatment groups. The DNA of FHV-1 was shed (120/133 [90%] samples; \( P = 0.038 \)) significantly less frequently in famciclovir-treated cats, compared with shedding (81/83 [98%] samples) in lactose-treated cats, when considering all shedding episodes for all cats throughout the study. Following logarithmic transformation of the data, FHV-1 DNA was detected significantly (\( P = 0.001 \)) less often in famciclovir-treated cats on day 4, compared with DNA detection in lactose-treated cats (Figure 7). Mean values for FHV-1 viral load (No. of FHV-1 RNA molecules/\( \mu \text{L} \)) were calculated by week because of the high interday and interindividual variability in these data sets. Following logarithmic transformation of the data, FHV-1 RNA was detected significantly (\( P = 0.017 \)) less often in famciclovir-treated than in lactose-treated cats during week 3 (Figure 8).

**Plasma penciclovir concentration**—In famciclovir-treated cats, mean trough penciclovir plasma concentrations at 8:00 AM and 2:00 PM were 0.52 ± 0.17 \( \mu \text{g/mL} \) and 1.4 ± 0.44 \( \mu \text{g/mL} \), respectively, whereas approximate peak concentrations at 11:00 AM and 3:00 PM were 2.0 ± 0.23 \( \mu \text{g/mL} \) and 2.1 ± 0.78 \( \mu \text{g/mL} \), respectively. Only 2 of 28 approximate peak values were greater than the target penciclovir plasma concentration of 3.5 \( \mu \text{g/mL} \) (Figure 9). Penciclovir was not detected in any samples obtained from lactose-treated cats.

**Discussion**

In the study reported here, we determined that the oral administration of famciclovir at a dosage of 90 mg/
kg 3 times daily to cats experimentally inoculated with FHV-1 was associated with significant reductions in total clinical disease score, histologic conjunctivitis score, serum anti–FHV-1 antibody titer, serum globulin concentration, FHV-1 DNA viral load detected in conjunctival cytobrush samples, incidence of FHV-1 RNA viral load detected at the ocular surface, and increased GCD in cats with primary herpetic disease. In addition, famciclovir-treated cats in the present study had an increase in body weight throughout the study, compared with results for lactose-treated cats, which had a decrease in body weight during the first 7 days after inoculation with FHV-1. Although food intake was not measured, this outcome was presumably caused by a decrease in the intake of food, water, or both because of anorexia and malaise as signs of clinical disease peaked. Importantly, no adverse clinical or clinicopathologic effects were observed in cats orally administered famciclovir at this dosage and frequency for 21 days. In contrast, oral administration of the pharmacologically related antiviral compound valacyclovir (60 mg/kg, q 6 h) in cats experimentally infected with FHV-1 resulted in more severe clinical signs of herpetic disease, severe bone marrow suppression and leukopenia, marked electrolyte abnormalities and crystalluria, dehydration, lethargy, and, ultimately, fatal coagulative necrosis of the renal tubular epithelium and centrilobular hepatic atrophy.15 Because famciclovir and valacyclovir are prodrugs and their administration is intended to improve the bioavailability of their effective metabolites penciclovir and acyclovir, respectively, analysis of the results of the study reported here indirectly suggests that penciclovir is more efficacious and less toxic than acyclovir is in cats. This hypothesis is supported by the observation that acyclovir-associated renal toxicosis secondary to crystallizing nephropathy in humans resolves when famciclovir is substituted for acyclovir.12

Although histologic examinations of liver or kidney tissues were not conducted in the present study, we observed no hematologic or serum or urine biochemical changes that suggest bone marrow, renal, hepatic, or other organ-specific toxicosis in any famciclovir-treated cat at any time point during the 21 days of 3-times-daily administration of famciclovir. However, all cats in this study had apparently normal renal, hepatic, and bone marrow functions prior to inclusion in the study and were healthy prior to inoculation with FHV-1. In addition, the most commonly reported adverse effects of famciclovir treatment in humans include urticaria, hallucinations, headaches, and confusion (especially in elderly humans),32 which would likely be more difficult to detect in animals. For these reasons, judicious use of this drug is recommended in client-owned cats, especially those with preexisting hepatic or renal insufficiency.

In the present study, serum globulin concentration increased in both treatment groups, as would be expected in naive animals inoculated with an infectious agent for the first time. However, the magnitude of this change was reduced in famciclovir-treated cats. In addition, their terminal anti–FHV-1 antibody titers were reduced, compared with the anti–FHV-1 antibody titers in lactose-treated cats. It seems likely that the lower serum globulin concentration observed in the famciclovir-treated cats was, in part, caused by a decrease in anti–FHV-1 antibody production. In addition, reductions were observed for both the amount and incidence of FHV-1 DNA detected in the conjunctival fornix of famciclovir-treated cats. Furthermore, famciclovir administration significantly decreased the amount of viral replication as measured by the detection of FHV-1 RNA. These results are similar to those for clinical trials in humans in which daily oral administration of famciclovir reduced detection of HSV-1 and HSV-2 DNA in patients with or without symptoms of genital herpes.31,32 Analyses of these data32,33 also suggest that the clinical effects observed in the study reported here were directly attributable to an antiviral effect of penciclovir and are consistent with analyses of in vitro data that suggest the antiviral effect of penciclovir against FHV-1 is approximately equivalent to that of antiviral drugs such as idoxuridine and cidofovir.12,13,17 Because FHV-1 is relatively unstable in the environment, latently infected cats represent the most important reservoir for FHV-1 infection of naive cats, and limitation of viral shedding in latently infected cats is presumed to be an important epidemiological tool for controlling the spread of FHV-1 in multiple-cat settings. Additional studies are required to determine whether data from the present study are applicable for treatment of recrudescent disease in cats naturally infected with FHV-1 and whether the administration of famciclovir reduces disease prevalence in at-risk cat populations. However, analysis of data from another study34 suggests that the administration of 62.5 mg of famciclovir (8 to 21 mg/kg, PO, q 12 to 24 h) to cats with spontaneous ocular disease or rhinosinusitis attributed to FHV-1 or administration of 125 mg of famciclovir (30 mg/kg, PO, q 8 h) to treat spontaneous herpetic dermatitis was associated with improvement in clinical signs of disease in some cats. However, investigators of that 10-cat study36 were not masked with regard to treatments, and a negative control group was not included.

Although in the present study we detected that the oral administration of famciclovir was associated with faster recovery of GCD, this value remained below the reported32 reference range for ≥ 3 weeks in both lactose-treated and famciclovir-treated cats. This observation is consistent with the findings of a study23 in which investigators reported that primary FHV-1 infection induces qualitative abnormalities in the tear film (measured via TFBUT and GCD) for ≥ 29 days, which was long after there was improvement in clinical signs of conjunctivitis. When analyzed together, data from that study23 and the study reported here suggest that mucin replacement treatment with products such as sodium hyaluronate should be considered in addition to administration of famciclovir for clinical management of FHV-1 infection in cats. In addition, cats in the famciclovir-treated and lactose-treated groups in the present study had corneal ulcers, and no significant difference in ulcer prevalence or duration was detected between treatment groups. Therefore, topical antimicrobial treatment to prevent secondary bacterial infection of ulcers may also be necessary in famciclovir-treated cats. Collectively, these data also reinforce the suggested need for appropriate diagnostic testing (including STT, TFBUT, and appli-
istration of the dose at 8:00 am. The peak plasma penciclovir concentration may have been measured after administration of 1 of the first 2 doses of the day (8:00 am, 2:00 pm, and 8:00 pm) produced dosage intervals that were not equal or similar to those in the other study. However, such an interstudy comparison may not be reliable because cats in that other study were administered a lower dose of famciclovir (9 to 16 mg/kg, q 8 h) for only 3 days. In addition, timing of famciclovir administration in the present study (8:00 AM, 2:00 PM, and 8:00 PM) produced dosage intervals that were not equal or similar to those in the other study, and samples to estimate peak plasma penciclovir concentrations in the present study were collected approximately 3 hours after the oral administration of famciclovir. The collection of samples at this time point may have been slightly before the time at which peak penciclovir plasma concentrations were achieved, as indicated by the time of peak plasma concentrations (3.8 ± 0.5 hours) reported in another study. However, such an interstudy comparison could account for this observation. Samples intended to measure approximate peak plasma penciclovir concentrations in the present study were collected approximately 3 hours after the oral administration of famciclovir. The collection of samples at this time point may have been slightly before the time at which peak penciclovir plasma concentrations were achieved, as indicated by the time of peak plasma concentrations (3.8 ± 0.5 hours) reported in another study.

Although a dosage of 90 mg of famciclovir/kg administered orally 3 times daily was remarkably efficacious in the study reported here, the approximate peak plasma penciclovir concentration (2 to 2.1 µg of penciclovir/mL) was less than the target concentration of 3.5 µg of penciclovir/mL that was based on analysis of in vitro data. Several explanations could account for this observation. Samples intended to measure approximate peak plasma penciclovir concentrations in the present study were collected approximately 3 hours after the oral administration of famciclovir. The collection of samples at this time point may have been slightly before the time at which peak penciclovir plasma concentrations were achieved, as indicated by the time of peak plasma concentrations (3.8 ± 0.5 hours) reported in another study. However, such an interstudy comparison may not be reliable because cats in that other study were administered a lower dose of famciclovir (9 to 16 mg/kg, q 8 h) for only 3 days. In addition, timing of famciclovir administration in the present study (8:00 AM, 2:00 PM, and 8:00 PM) produced dosage intervals that were not equal or similar to those in the other study, and samples to estimate peak plasma penciclovir concentrations in the present study were collected following the administration of 1 of the first 2 doses of the day at 8:00 AM and 2:00 PM. A higher peak penciclovir concentration may have been measured after administration of the dose at 8:00 PM because this administration was only 6 hours after administration of the preceding dose. This dosing schedule was chosen for convenience and because it likely simulates many clinical situations in which owners may be unable to administer 3 doses daily at regular 8-hour intervals. The efficacy of famciclovir at lower-than-expected plasma penciclovir concentrations observed in the present study may also relate to the pharmacokinetics of famciclovir and penciclovir in cats, which appear to be complex, nonlinear absorption or metabolism of famciclovir, nonlinear excretion of penciclovir, or any combination of these 3. When comparing pharmacokinetic data from another study with those the study reported here, it appears that a 5- to 10-fold increase in the dosage of famciclovir was associated with only a 3-fold increase in the Cmax of penciclovir. In addition, the peak plasma penciclovir concentration predicted with the linear model used to determine the dosage and frequency of administration for the present study underestimated Cmax (Figure 9). Collectively, analysis of these data provides further evidence that it is likely that famciclovir absorption, famciclovir metabolism, penciclovir elimination, or a combination thereof is a nonlinear phenomenon in cats. Further pharmacokinetic studies that utilize IV administration of penciclovir and oral administration of alternate dosages of famciclovir will be required to better understand the complex pharmacokinetics of famciclovir and penciclovir in cats.

A possible explanation for famciclovir efficacy at lower-than-expected plasma penciclovir concentrations in the present study is that famciclovi or one of its intermediate metabolites may have antiviral activity against FHV-1 that is additive to that attributed to penciclovir. This explanation appears less likely because of the specific structure-activity relationship required for antiviral activity of a nucleoside analog such as penciclovir. However, further in vitro studies are required to determine whether the potential antiviral activity of famciclovir or intermediate metabolites contributes to the anti-FHV-1 efficacy of penciclovir. Finally, it is possible that results of a previous study represent an overestimation of the IC50 of penciclovir for FHV-1. In subsequent in vitro studies, investigators have reported a lower IC50 of penciclovir for FHV-1 (1.2 to 1.6 µM [0.30 to 0.41 µg/mL]), compared with the IC50 (13.9 µM [3.5 µg/mL]) reported in the aforementioned study. This may in part indicate variation in study methods, such as differences in the strain of FHV-1 tested and the viral titration method used. However, investigators who evaluated the effect of viral strain on the efficacy of antiviral drugs, but not penciclovir specifically, reported that the strain of FHV-1 was not an important factor for antiviral susceptibility. In addition, the same FHV-1 isolate (strain 727) was used for the in vitro estimation of IC50 of penciclovir and for the in vivo estimation reported in the present study. Any one of these proposed mechanisms may also help explain the reported efficacy in cats presumed to be undergoing recrudescent herpetic disease and administered famciclovir as infrequently as once daily and at a dosage, which was approximately 3- to 11-fold less than the dosage reported in the present study.

Although further investigation of the pharmacokinetics of famciclovir and penciclovir as well as the mode of action of these drugs in cats is warranted to define the optimal dose, results for the study reported here suggest that 90 mg of famciclovir/kg administered orally 3 times daily to nonvaccinated specific-pathogen–free cats experimentally infected with FHV-1 results in significant improvement in numerous systemic, ophthalmic, clinicopathologic, virologic, and histologic variables, compared with results for these variables in lactose-treated cats. Furthermore, famciclovir administration was tolerated well in these cats and was not associated with clinically or clinicopathologically detectable adverse effects. However, the tear film of famciclovir-treated cats remained abnormal for the duration of the study, and corneal ulcers were not reduced by famciclovir administration. Therefore, adjunctive topical mucinomimetic and antimicrobial treatments also may be warranted in FHV-1–infected cats treated by administration of famciclovir.
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