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Pilot Study of the Safety and Tolerability of a Subconjunctival Penciclovir Implant in Cats Experimentally Infected with Herpesvirus

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Abstract

Purpose: To assess safety and tolerability of a subconjunctival penciclovir implant in cats infected with feline herpesvirus type 1 (FHV-1).

Methods: Subconjunctival blank ($n=4$ cats) or penciclovir-impregnated ($n=6$) silicone implants were placed bilaterally in 10 normal, FHV-1-naive cats 7–8 days before viral inoculation. Outcomes included disease score, FHV-1 serology, conjunctival viral load, Schirmer tear tests (STT), tear film break-up times (TFBUTs), conjunctival histology, goblet cell density (GCD), body weight, tear and plasma penciclovir concentration, and corneal ulcer evaluation.

Results: Both groups had similar clinical and histologic disease scores, STT values, TFBUTs, GCD, FHV-1 titers, viral loads, and body weight changes. No ocular or systemic signs of toxicity were noted. Tear penciclovir concentration varied widely among cats and across time points. Tear penciclovir concentrations exceeded the lowest published half maximal inhibitory concentration (IC_{50}) in 5/6 treated cats. Plasma penciclovir concentrations remained below 10 ng/mL. Cats with higher tear penciclovir concentrations at inoculation and/or time of peak disease had fewer corneal ulcers than cats in which tear penciclovir concentrations were inconsistent, low, or unrecordable.

Conclusions: Subconjunctival blank and penciclovir-impregnated implants were well tolerated at the ocular surface and not associated with systemic toxicity, adverse effect, or appreciable plasma penciclovir concentrations. Tear penciclovir concentrations $>IC_{50}$ were sometimes achieved, especially during burst release soon after implant placement. Further study is necessary to determine efficacy of locally delivered penciclovir when penciclovir concentration is consistently maintained above IC_{50}. This will be especially useful in patients unable to receive systemic therapy.

Keywords: subconjunctival implant, cat, herpes virus, penciclovir, preclinical pharmacology

Introduction

Herpes simplex virus type 1 (HSV-1) and feline herpesvirus type 1 (FHV-1) are both alphaherpesviruses[1] that are ubiquitous within their natural host populations,[2–4] replicate rapidly within and lyse epithelial cells,[5,6] produce pathognomonic dendritic corneal ulcers,[7,8] are variably responsive to antiviral compounds,[9] establish lifelong neural latency,[10,11] and periodically reactivate, causing epidemiologically important, blinding recrudescent disease.[12,13]

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Given these similarities, cats infected with FHV-1 represent an important model for studying the pathogenesis of and therapies for herpetic disease.[14]

Many antiviral drugs for the treatment of herpetic disease are unsafe when administered systemically, such that only local application is possible.[9,15] Even when topically administered at the ocular surface, many antiviral drugs can cause corneoconjunctival toxicity and require frequent application, which may result in noncompliance and may also lead to development of drug-resistant strains. Therefore, alternative methods for delivery of antiviral drugs at the ocular surface are warranted. This would be of particular value in patients unable to tolerate or insufficiently compliant with multiple daily administration of topical or oral medications, or for those in whom comorbidities may render systemic medication less safe. This has stimulated extensive investigation of drug release kinetics at the ocular surface from a wide range of delivery devices.[16–23]

Oral famciclovir, a penciclovir prodrug, is widely prescribed for humans[24,25] and cats[26–28] with herpetic disease. While some indications for use may differ between the 2 species, famciclovir is prescribed for herpetic keratitis in both species.[26–31] In this study, we assessed the safety and tolerability of delivering penciclovir at the corneal surface from a nonbiodegradable subconjunctival implant as a means to overcome concerns regarding systemic drug toxicity and patient compliance. This implant was previously used to deliver acyclovir subcutaneously to mice inoculated with HSV-1,[32] and, in *in vitro* experiments, it has been demonstrated to release penciclovir and suppress replication of FHV-1.[33] However, to the authors' knowledge, there are no reports of use of penciclovir-containing implants for treatment of herpetic ocular surface disease in any animals experimentally infected with their species-specific herpesvirus. As such, this study is exploratory in nature and provides some early descriptive observations regarding the safety and tolerability of penciclovir delivered by this implant in a novel species and at a novel tissue site. In particular, we focused on safety and tolerability of the implant and subconjunctivally delivered penciclovir, as well as assessment of penciclovir release from the implant, and maintenance of half maximal inhibitory concentration (IC_{50}) in tears when this implant was placed subconjunctivally in cats inoculated with FHV-1. Our goal was that these pilot data might be used to decide whether further refinements of this implant were worthwhile.

Methods

Experimental design

This study was conducted over 38 days and included surgical implant placement (randomized to occur on 1 of 2 days) followed by a 7–8 day observation period, simultaneous inoculation of all cats with FHV-1, a 28-day postinoculation observation period, implant removal, and adoption of all cats into private homes (Fig. 1). All cats were managed in accordance with the Association for Research in Vision and Ophthalmology Statement for Use of Animals in Ophthalmic and Vision Research, and all procedures were approved by the Institutional Animal Care and Use Committee (Protocol No. 15664) at the University of California-Davis. Ten intact female, unvaccinated, specific pathogen free, domestic short-haired cats were used. Their median (range) age and body weight were 6.6 (5.5–7) months and 2.8 (2.1–3.3) kg, respectively. Cats were group-housed at constant ambient temperature (21°C ± 2°C) and light-to-dark cycle ratio (14:10 h), and had *ad libitum* access to fresh water and a commercially prepared dry diet.

Before study entry, all cats were verified as normal based upon results of general physical examination, slit lamp biomicroscopy before and after pupil dilation, and binocular indirect ophthalmoscopy after pupil dilation, all performed by a board-certified veterinary ophthalmologist (D.J.M.). In addition, all cats had normal results of a complete blood count (CBC), serum biochemistry panel, and urinalysis; and serum from all cats was verified to be free of antibodies to feline immunodeficiency virus and antigens of feline leukemia virus (HESKA Corporation and IDEXX SNAP FIV/FeLV Combo Test)—2 retroviruses known to alter immunocompetence in cats.

Cylindrical (15 × 2 mm) silicone implants with or without 33% (w/w%) penciclovir were prepared as described.[32] Cats were randomized (by drawing of cards) to receive blank (4 cats) or penciclovir-containing (6 cats) implants surgically placed in the subconjunctival space by a veterinary ophthalmology resident (S.M.T.) supervised by a board-certified veterinary ophthalmologist (D.J.M.). Until all data were analyzed, only 1 investigator uninvolved in data collection (H.K.-F.) was aware of group assignment; all remaining investigators were masked as to implant group. Cats were randomized to undergo surgical implant placement on 1 of 2 days (day Negative 7 or 8) so that all surgeries could be performed by a single surgeon (S.M.T.). All cats received the same implant type in both eyes. Briefly, cats were placed under general anesthesia and the superior bulbar conjunctiva of each eye was incised for about 10–15 mm ~5 mm posterior to and parallel with the superior corneoscleral limbus. The conjunctiva was then undermined and reflected to create a fornix-based subconjunctival pocket. The implant was secured to the episclera and sclera with a single, centrally placed 4-0 nylon suture. The conjunctiva was closed using 6-0 polyglactin in a simple continuous pattern. Median (range) surgical time for both eyes was 35 (30–45) min. Neomycin-polymyxin-bacitracin ophthalmic ointment was placed in both eyes twice daily for 5–6 days following implant placement. Antibiotic use was discontinued in all cats for 48 h before viral inoculation.

To assess implant tolerability and allow surgical irritation to subside before inoculation, all cats were observed for 7 or 8 days following implantation as determined by randomized day of implant placement. All cats were then simultaneously inoculated with FHV-1 on day 0. Briefly, all cats were inoculated in both conjunctival fornices and both nares with a total of 1×10^7 plaque-forming units (pfu) of FHV-1, strain 727, passage 11.[26] Cats then were monitored and sampled as described below for 28 days following inoculation, at which time, clinical disease caused by this inoculum is typically self-limiting.[26,34] To assess for any lasting adverse effects from herpetic infection or implant placement, a complete ophthalmic examination as described at study entry was repeated by the same veterinary ophthalmologist (D.J.M.) on day 28 or 29, and both implants were removed from all cats under general anesthesia by a single surgeon (D.J.M.) unaware of implant group. Briefly, a small incision was made in the dorsal bulbar conjunctiva and the nylon suture

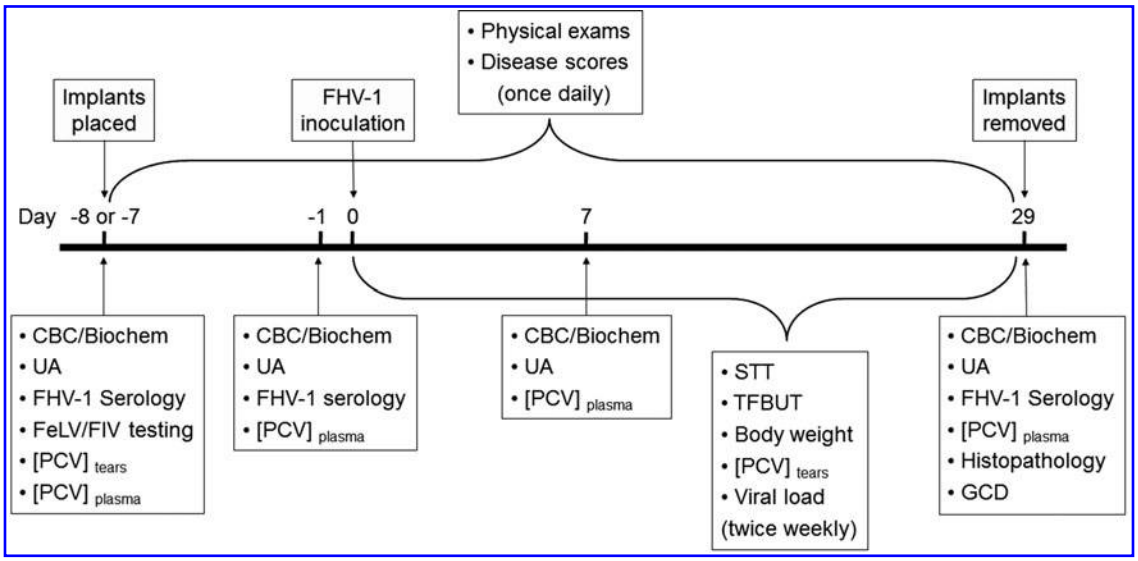

FIG. 1. Experimental design for study of 10 cats in which blank (4 cats) or penciclovir-containing implants (6 cats) were placed subconjunctivally on day Negative 8 or Negative 7 and removed on day 29. All cats were inoculated with FHV-1 on day 0. Multiple outcomes were assessed at the described intervals throughout the study. CBC/Biochem, complete blood count and serum biochemistry analysis; FeLV, feline leukemia virus; FHV-1, feline herpesvirus type 1; FIV, feline immunodeficiency virus; GCD, conjunctival goblet cell density; [PCV], penciclovir concentration; STT, Schirmer tear test; TFBUT, tear film break-up time; UA, urinalysis.

was cut and removed along with the implant. To assess histologic character and severity of conjunctivitis, as well as to enumerate conjunctival goblet cell density (GCD), an $\sim$3-mm^3 sample of bulbar conjunctiva and subconjunctiva overlying the implant in each eye was removed and placed immediately into neutral-buffered 10% formalin, routinely processed, and stained with hematoxylin and eosin (HE), and by using the periodic acid Schiff (PAS) method. The conjunctival rent due to implant removal and biopsy was allowed to heal by secondary intention. Neomycin-polymyxin-bacitracin ophthalmic ointment was placed in both eyes twice daily for 7–10 days following implant removal, and all cats were vaccinated, microchipped, spayed, and adopted into private homes.

Disease course and virological assessment

So as to characterize disease severity, clinical signs of ocular and upper respiratory tract disease were scored once daily throughout the experimental period by a single trained investigator (J.C.C.) using a previously reported system.[35] Ocular disease scoring included evaluation of conjunctivitis [scored 0 (absent) through 3 (severe)], blepharospasm [scored 0 (absent) through 4 (lids completely closed)], and ocular discharge [scored 0 (absent) through 3 (marked mucopurulent/serosanguineous)]. Upper respiratory disease scoring included sneezing [scored as 0 (absent) or 1 (present)] and nasal discharge [scored from 0 (absent) through 3 (marked mucopurulent)]. Scores were summed to create total clinical disease scores, with the maximum possible disease score being 24.

To quantify FHV-1 DNA, Dacron swabs were collected bilaterally from conjunctival fornices, as described,[35] 3 days before inoculation (ie, day Negative 3) and then every 4 days after inoculation through day 29. Swabs were collected following induction of surface anesthesia using 0.5% proparacaine hydrochloride ophthalmic solution applied from individual bottles, each assigned to 1 cat so as to minimize contamination. Swabs were stored at −20°C until nucleic acid extraction, performed according to the manufacturer's protocol (QIAmp DNA Micro Kit; Qiagen), except swabs were shaken in extraction buffer (55°C for 3 h), and extracts were loaded onto purification columns in 2 successive spins. After elution in 30 μL of buffer, nucleic acids were stored at −20°C until quantification of the FHV-1 thymidine kinase gene in triplicate using real-time quantitative polymerase chain reaction (qPCR) (Step One Real-Time PCR system; Applied Biosciences) with a commercially available kit (GoTaq qPCR Master Mix, Cat #A6001; Promega) as described,[36] except that 34 cycles were run in a final volume of 10 μL in 48-well reaction plates (MicroAmp 48-well reaction plates and Optical Film; Applied Biosystems). Standard curves were generated using known dilutions of virus (pfu/mL), and used to convert Ct values to approximate viral titer (pfu/mL) for each sample.

To demonstrate that all cats were naive to FHV-1 and to prove seroconversion, serum was collected on day Negative 8 or Negative 7, day 0, and day 29, and was assessed for presence of antibodies to a recombinant FHV-1 antigen using a commercially available ELISA (Specialized Infectious Disease Laboratory, Colorado State University).[37] Serum antibody titers were estimated by comparing mean absorbance values of each sample to a standard curve generated from positive and negative control samples.

Ocular and systemic safety

To assess ocular safety and tolerance of the implant and of penciclovir before and after FHV-1 inoculation, Schirmer tear tests (STT) using tear strips (HAAG-STREIT Schirmer Tear Test Strips Lot No. 23670) from the same lot number,[38] tear film break-up times (TFBUTs), and

slit lamp biomicroscopy following application of fluorescein to the ocular surface were performed every 3 or 4 days by 1 trained investigator (J.C.C.) as described.[39] All HE- and PAS-stained conjunctival sections were examined by a board-certified veterinary pathologist (C.M.R.); inflammation was characterized and scored, and GCD was calculated as described.[26,39]

To assess systemic safety and tolerance of the ocular implant and of penciclovir before and after FHV-1 inoculation, body weight of all cats was recorded twice weekly, and body temperature, and respiratory and heart rates were recorded once daily. Results of CBC, serum biochemical analysis, and urinalysis were obtained from blood collected by jugular or cephalic venipuncture, and from urine collected by cystocentesis at implant placement (day Negative 8 or Negative 7), 1 day before inoculation (day Negative 1), and at the time of approximate peak clinical disease (day 8) and implant removal (day 29).

Penciclovir quantification

Tears were collected by placing STT strips in the ventral conjunctival fornix for 1 min as described.[40] Tears then were eluted from the STT strips, and penciclovir concentration subsequently determined by liquid chromatography-mass spectrometry (LC-MS).[40] Penciclovir concentration was standardized per milliliter of tear using mass, determined by using the weight of strip before and after tear collection, and volume, assuming that 1 g and 1 mL of tears were equivalent. For plasma penciclovir analysis, blood was collected into lithium heparin tubes on day Negative 8 or Negative 7, day Negative 1, and day 29, and was stored on ice for 10–30 min before centrifugation at 1,000 *g* for 7–10 min. Plasma was separated and stored at −20°C. Penciclovir in plasma and tear samples was quantified using LC-MS as described.[40,41] The limits of quantitation for plasma and tears were 0.5 and 0.01 ng/mL, respectively.

Data analysis

Each eye of each cat was considered independently for analysis of STT results, TFBUT, presence of corneal ulcers, histologic conjunctivitis score, histologic conjunctival inflammation, and GCD. Viral load and tear penciclovir concentration for each cat were analyzed using the mean value from both eyes. Normally distributed data (as identified using the Shapiro-Wilk test) are presented as mean ± SD (standard deviation); non-normally distributed and ordinal data are reported as median and interquartile range (IQR). Because the implant contained the drug, and because neither drug nor implant had been tested previously in cats, consideration of the independent and joint effects of implant and drug was not possible, and simultaneous assessment of these factors rendered inferential statistical analysis unreliable.

Results

Disease course and virological assessment

Irrespective of implant type, all cats developed mild conjunctivitis that peaked about 2 days following implantation, and persisted until inoculation (Fig. 2). Following inoculation, all cats developed fever and typical clinical signs for this inoculum in FHV-1-naive cats.[26] Total clinical disease scores peaked over a 4-day period (days 6 through 9) for cats with blank implants versus a 2-day period (days 7 and 8) for cats with penciclovir-containing implants (Fig. 2). All cats were seronegative to FHV-1 on days Negative 8, Negative 7, 0, and 7, but had seroconverted by day 29 (titer range 1:64–1:2,048). Viral DNA was consistently detected in all blank- and penciclovir-implanted eyes at each of the sampling points following inoculation (Fig. 3).

Ocular and systemic safety

In both implant groups, mean STT values increased 3–4 days following implant placement, returned to approximate baseline values at inoculation, and then were relatively stable for the remainder of the study (Fig. 4A). Considering all 200 STT measurements in both groups, only 10 were below the reference range, and all, but 3 of these were within 2 mm/min of normal.[42] Five of the 7 abnormal values were from 1 cat with blank implants. In both implant groups, mean TFBUTs remained above normal[42] until peak clinical disease, when they decreased steadily to a minimum at day 24, which was below the reference range (Fig. 4B). Considering all 200 TFBUT measurements in both groups, 47 were below, but within 7.3 s of normal[42]; 32 of these occurred between day 15 and 23. All penciclovir- and blank-implanted cats had an abnormal TFBUT reading on at least 1 occasion during the study.

At surgical removal, 1 of 8 blank implants and 1 of 12 penciclovir implants (both from right eyes) were unable to be located in the subconjunctival space. Considering all biopsies collectively, histologic assessment of bulbar conjunctiva at implant removal revealed mild generalized conjunctivitis, small nodular aggregates of macrophages and neutrophils (considered likely suture-associated inflammation), or sometimes both in all cats (Fig. 5A, B). Inflammation in eyes with a blank implant was lymphoplasmacytic or mixed (4 eyes each). Inflammation in eyes with a penciclovir implant was absent (3 eyes), lymphoplasmacytic (5 eyes), or mixed (4 eyes). Median (range) histological conjunctivitis scores were similar in eyes with blank [1 (1–2)] or penciclovir [1 (0–3)] implants. Median (range) suture-associated inflammation score was 2 (0–3) for eyes with blank or penciclovir implants. Median (IQR) GCD for cats with blank [7 (1–25)] or penciclovir [12 (4–18)] implants was below the lower limit of the reference range (Fig. 5C, D).[43] For cats with blank implants, GCD was below the reference range for 6 eyes (4 cats), and above the reference range for 2 eyes (2 cats).[43] For cats with penciclovir implants, GCD was below the reference range for 6 eyes (6 cats), and above the reference range for 1 eye (1 cat).[43]

Body weight remained relatively constant throughout the study period in both groups, except at the time of peak disease when mean ± SD body weight decreased temporarily in cats with a blank (6.4% ± 0.9%) or penciclovir (3.8% ± 1.3%) implant. Elevated body temperature and heart and respiratory rates developed around time of peak disease in all cats irrespective of implant type, but were within normal limits for all cats at other times. No clinical signs of systemic toxicity attributable to blank or penciclovir implants were noted at any time. Likewise, results of CBC,

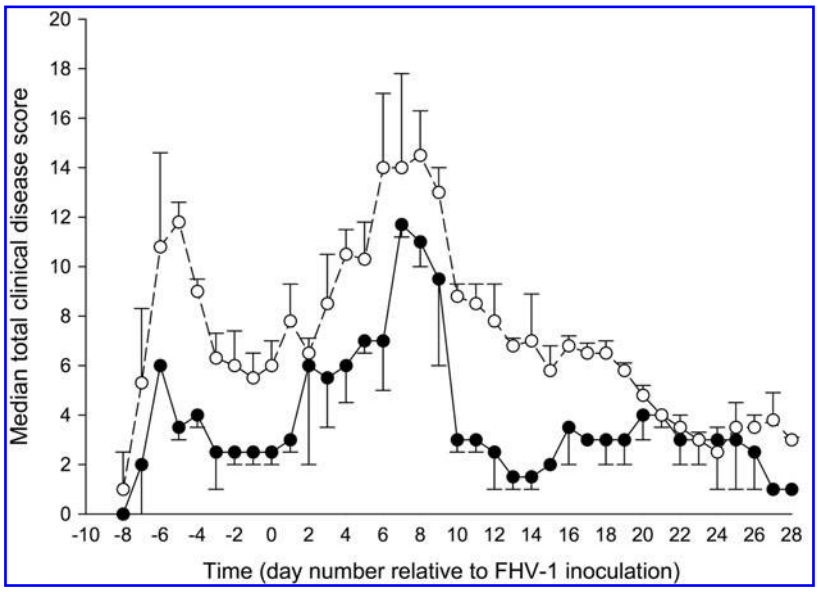

FIG. 2. Median ± interquartile range total clinical disease scores for 10 cats in which blank (*open circles*, *dashed line*; $n=4$) or penciclovir-containing (*black circles*, *solid line*; $n=6$) implants were placed subconjunctivally on day Negative 8 or Negative 7 and removed on day 29. All cats were inoculated with FHV-1 on day 0. Total clinical disease score was defined as the sum of all scores for conjunctivitis, blepharospasm, ocular discharge, sneezing, and nasal discharge, and was recorded daily. Minimum and maximum possible disease scores were 0 and 24, respectively.

serum biochemical analysis, and urinalysis revealed no evidence of hepatic, renal, or bone marrow impairment.

Penciclovir quantification

Penciclovir was detected in tears and plasma of all cats with a penciclovir implant; however, tear penciclovir concentration varied widely among cats and across time points (Fig. 6). Tear penciclovir concentrations were highest within 1 week following implant placement in all cats, and exceeded the lowest published IC_{50} for FHV-1[44] (304 ng/mL) in 5/6 cats. Subsequently, tear penciclovir concentration waned to low or undetectable concentrations for the remainder of the study in 3/6 cats. For the eye in which a penciclovir-impregnated implant was not found at study end, penciclovir was not detected beyond day 15. Total tear penciclovir over the study period was estimated by area-under-the-curve analysis using all time points assayed throughout the study. Average daily tear penciclovir concentration exceeded the target IC_{50} in only 1 cat in which it was 425 ng/mL/day. Plasma penciclovir concentrations never exceeded 10 ng/mL in any cat.

Corneal ulceration

Although corneal ulcers were seen in some cats from both implant groups, they occurred with greater frequency, severity, and duration in the blank- versus penciclovir-implant group (Table 1). Dendritic corneal ulcers were observed in 3/4 cats with blank implants and 3/6 cats with penciclovir implants. In the 3 blank-implanted cats, ulcers were detected on 11 occasions between days 11 and 27, and persisted for no more than 4, 12, or 16 days in each cat, respectively. Because corneal ulceration was assessed only once every 3 or 4 days, ulcer duration could not be calculated more accurately than this. Ulcers were bilateral on 5 occasions in 1

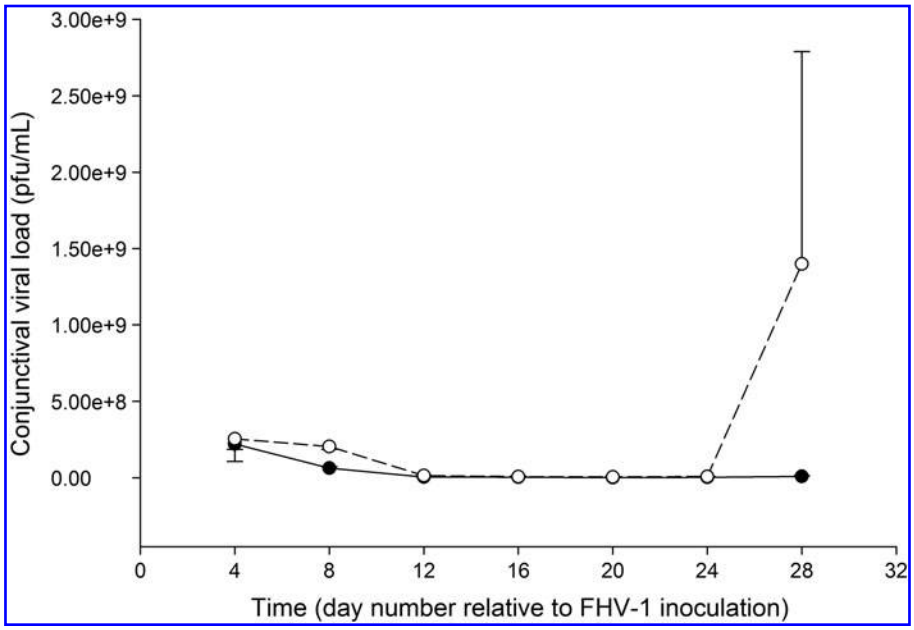

FIG. 3. Mean ± SD conjunctival viral load (plaque-forming units/mL) for 10 cats in which blank-containing (*open circles*, *dotted line*; $n=4$) or penciclovir-containing (*black circles*, *solid line*; $n=6$) implants were placed subconjunctivally on day Negative 8 or Negative 7 and removed on day 29. All cats were inoculated with FHV-1 on day 0. SD, standard deviation.

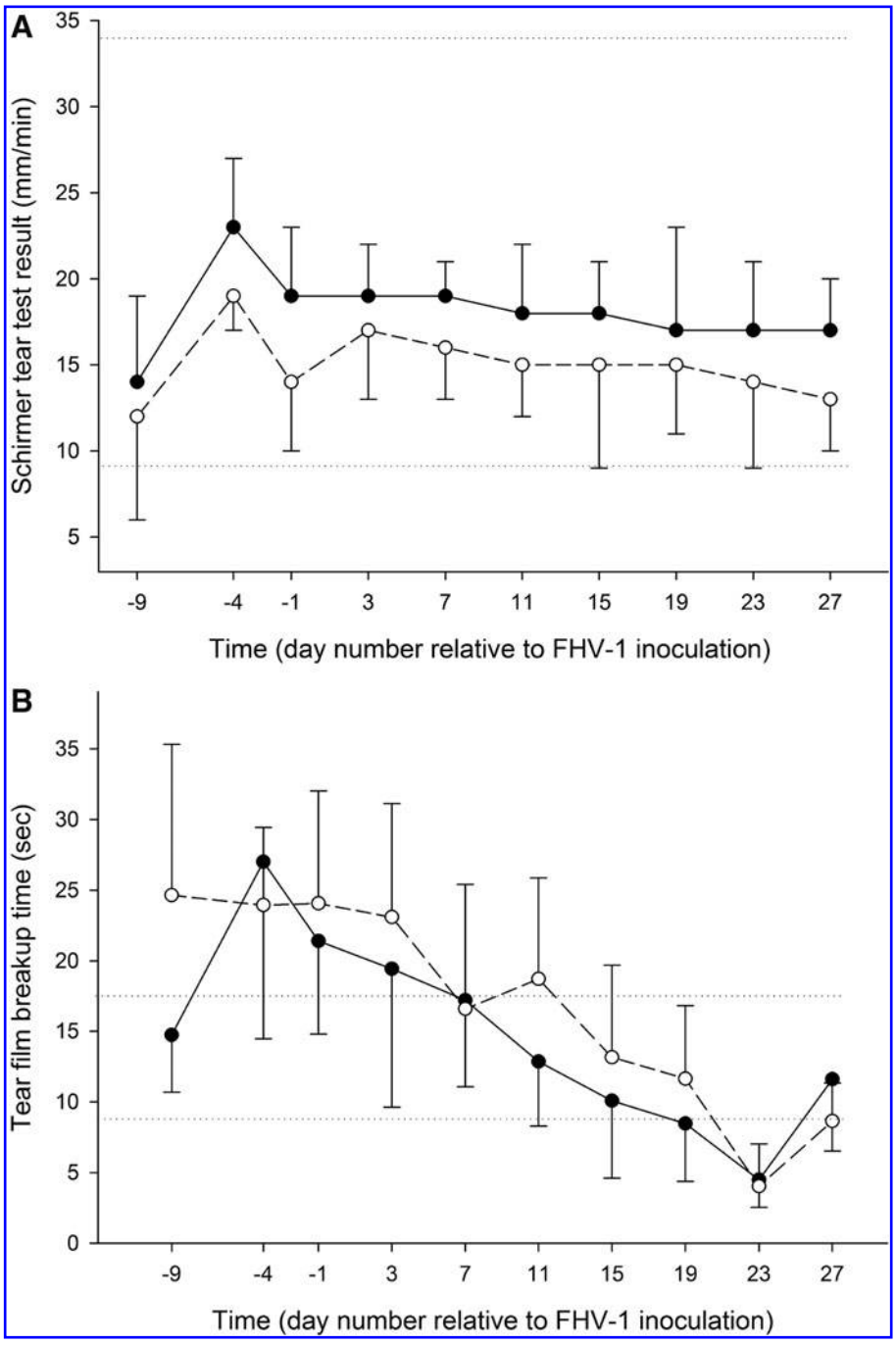

FIG. 4. Mean ± SD STT values (mm/min; **A)** and TFBUTs (s; **B)** for 10 cats in which blank (*open circles*, *dashed line*; $n = 4$) or penciclovir-containing (*black circles*, *solid line*; $n = 6$) implants were placed subconjunctivally on day Negative 8 or Negative 7 and removed on day 29. All cats were inoculated with FHV-1 on day 0. *Horizontal dotted lines* indicate the reference ranges for both tests.[37]

cat, and on 1 occasion in 1 cat. In the 3 penciclovir-implanted cats, ulcers were detected on 8 occasions between days 11 and 27, and persisted for no more than 1, 9, or 10 days in each cat, respectively. Ulcers in penciclovir-implanted cats were always unilateral, except in 1 cat on 1 occasion.

Simultaneous analysis of corneal ulceration and tear penciclovir concentration for all penciclovir- and blank-implanted cats revealed that fewer corneal ulcers were observed when tear penciclovir concentration exceeded the lowest published IC_{50} of penciclovir for FHV-1.[44] In 5/6 penciclovir-implanted cats, tear penciclovir concentration exceeded this IC_{50} on at least 1 occasion during the study—3 cats at the time of inoculation, 1 cat at both inoculation and peak disease, and in the last, at later time points in the study (days 11 and 19). Four of these 5 cats had no or infrequent ulceration (Fig. 7A). Tear penciclovir concentrations in the sixth penciclovir-treated cat never exceeded the target IC_{50}, and this cat experienced frequent ulceration (Fig. 7B). Penciclovir was never detected in the tears of the 4 blank-implanted cats, 3 of which had frequent and often bilateral corneal ulceration (Fig. 7C).

Discussion

In this exploratory pilot study, we have made some initial observations regarding the safety and tolerability of penciclovir delivered by a subconjunctival implant in a feline

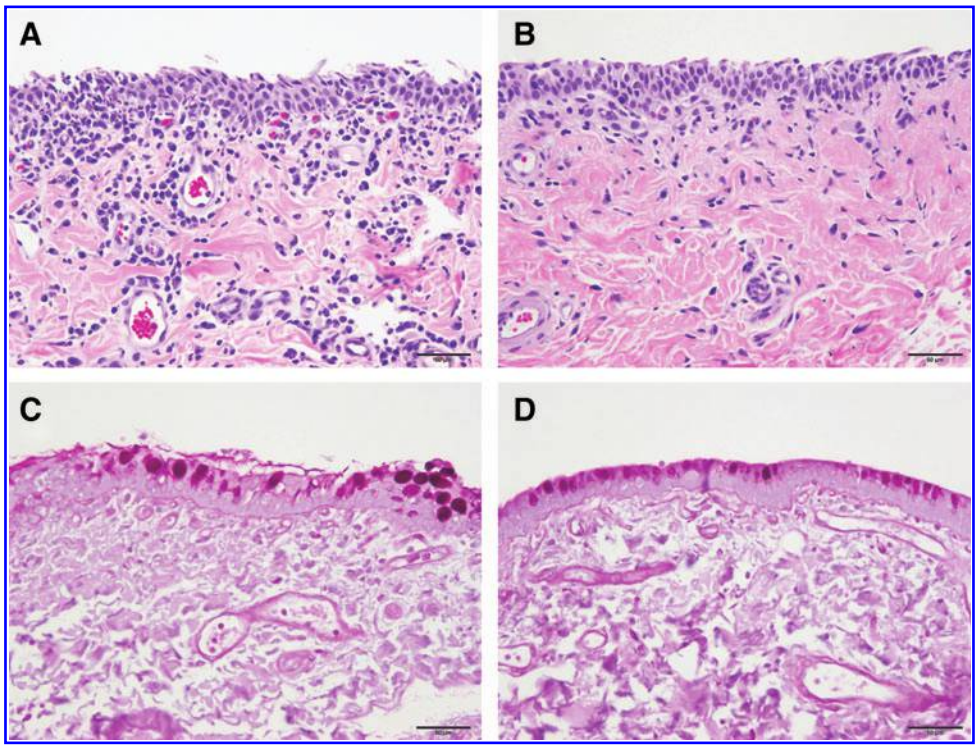

FIG. 5. Representative photomicrographs of feline bulbar conjunctiva that had been overlying blank **(A, C)** or penciclovir-containing **(B, D)** implants. Sections were stained with hematoxylin eosin **(A, B)** or using the periodic acid Schiff method to highlight conjunctival goblet cells **(C, D)**. Biopsies were collected 37–38 days following implant placement (ie, 29 days following inoculation with feline herpesvirus). Scale bar = 50 μm.

ocular herpetic disease model. As such, this study represented assessment of a novel implant, in a novel location, with a novel antiviral drug for this feline herpetic infection model. Therefore, consideration of potential treatment effects of the drug independent from those of the implant was not possible, and, like all exploratory studies, inferential statistical analysis would have been unreliable,[45] and was not performed. Instead, likely feasibility of penciclovir delivery during herpetic disease was assessed by (1) toxicity screening using biochemical and ocular normative ranges as limits, (2) observation of implant tolerability and retention, and (3) determining whether, when, and for how long the lowest published IC_{50} for FHV-1[44] was exceeded within the tears.

Ideally, a group of cats that had received the implants, but remained uninfected would have been included in this study; however that was not feasible in this small pilot study. Instead, we compared data in infected and implanted cats with those data gathered in the same cats during the initial 1-week period following implantation, but before infection. In addition, we made qualitative comparisons of data from this study generated in cats with the blank or penciclovir implant with data from other studies from our group using the same inoculum in cats treated with the oral penciclovir prodrug—famciclovir—or placebo.[26,39] In these prior studies, animals receiving no treatment exhibited a reliable disease course and reproducible changes in ocular parameters, consistent with data from others who have demonstrated that the feline

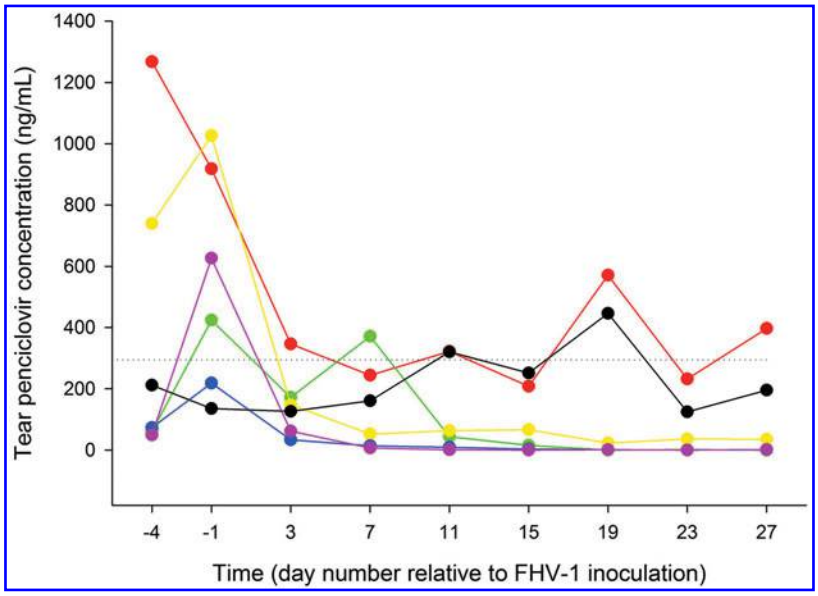

FIG. 6. Tear penciclovir concentrations for the 6 cats in which penciclovir-containing implants were placed subconjunctivally on day Negative 8 or Negative 7 and removed on day 29. All cats were inoculated with FHV-1 on day 0. Each *colored line* represents averaged data from right and left eyes of an individual cat. The *horizontal dashed line* represents the lowest published IC_{50} of penciclovir for FHV-1 (304 ng/mL).[39] IC_{50}, half maximal inhibitory concentration.

Table 1. Frequency of Corneal Ulceration in 10 Cats in Which Blank (4 Cats) or Penciclovir-Containing Implants (6 Cats) Were Placed Subconjunctivally on Day Negative 8 or Negative 7 and Removed on Day 29

	Day relative to FHV-1 inoculation (day 0)									
Cat No.	*−7 or −8*	*−4*	*−1*	*3*	*7*	*11*	*15*	*19*	*23*	*27*
Blank implants										
1										
2						+	+			
3						++	++	++	++	++
4							+	++	+	+
Penciclovir implants										
1						+				
2								++	+	+
3										
4						+		+	+	+
5										
6										

All cats were inoculated with FHV-1 on day 0, and following ophthalmic application of fluorescein were observed on 10 occasions for the presence of unilateral (+) or bilateral (++) corneal ulcers. Considering corneas of the 4 blank-implanted cats, 3 cats experienced at least 1 corneal ulcer. Ulcers were bilateral in 2 of these 3 cats and on 6 of the 11 days on which ulcers were noted in this group. Corneal ulcerative events (defined as days on which any eye was ulcerated) were recorded on 17 of the 80 observations. By comparison, only 3/6 cats with a penciclovir implant experienced a corneal ulcer. Ulcers were bilateral in only 1 cat, and on only 1 day. Corneal ulcerative events were recorded on only 9 of the 120 observations.

FHV-1, feline herpesvirus type 1.

FHV-1 model is reliable.[46] Data from cats in this study with blank implants were not obviously different from data from untreated cats in previous studies. This permitted us to hypothesize that differences seen in this pilot study between blank- and penciclovir-implanted cats were likely associated directly with the implant or penciclovir; however, other explanations are possible and should be tested in future studies in which uninfected implanted cats are assessed. Implants from 2 eyes could not be located at the time of removal, presumably as a result of migration out of the subconjunctival space. Although loss of the implants was not observed during the study, loss of the penciclovir implant was likely toward the end of the study since penciclovir was detected in the tears of the right eye until the last 3 time points. Despite penciclovir being undetectable in the tears at these last 3 time points, penciclovir concentrations for this cat exceeded IC_{50} at the time of peak disease, and no ulcers were present in either eye throughout the study. No ill effect directly attributable to implant loss was noted.

Ocular safety and tolerability of penciclovir and the implant were further assessed by frequent evaluation of multiple clinical parameters, including clinical and histologic disease scores, STT, TFBUT, and GCD. Relative to blank-implanted cats, penciclovir-implanted cats had similar clinical disease scores, STT values, and TFBUTs throughout the study, and GCD and histologic disease scores at study end. In addition, individual and group mean STT values remained largely within reference ranges for both groups. However, individual and mean TFBUTs fell below normal limits[42] after inoculation, and GCD was abnormally low[43] at study end. These abnormalities are temporally and quantitatively very similar to data generated in untreated cats infected with this inoculum of FHV-1 in other studies.[26,39] Taken together, these data suggest that an obvious effect at the ocular surface attributable to the implant or penciclovir was not observed, and that penciclovir, itself, did not add to implant-associated irritation. These observations in combination with the low penciclovir concentrations 1 week after implantation suggest that insufficient penciclovir may have been loaded into the implant and/or drug release of drug from the implant was inadequate *in vivo*. These implants contained 33% (w/w%) penciclovir and were designed to deliver this drug to the ocular surface at 5 μg/day for a cat's lifespan (~15 years).[32] However, up to 55% (w/w%) penciclovir can be loaded into these silicone implants (Margulies, unpublished data), and further *in vitro* and *in vivo* studies with higher concentrations are warranted.

For about 1 week immediately following surgical implantation, but before viral inoculation, cats were observed for response to the device itself. Despite an expected period of initial irritation following surgical implantation, neither the peak nor the duration of the clinical disease course was notably different from that seen in other studies using this inoculum.[26,39] In addition, median scores for blank and penciclovir implants were similar to each other within 2 days after inoculation and before peak disease. Inclusion of a sham-operated group would be necessary to better determine inflammation attributable to the implant itself, and whether such inflammation exerted an antiviral effect or compounded viral inflammation. However, in this study, viral loads tended to be lower in cats in which the penciclovir IC_{50} was achieved than in cats with blank implants; and median disease scores for the blank implant group in this study were similar to those seen in untreated cats in previous studies.[26,39]

Systemic safety and tolerability of penciclovir and the implant were assessed by multiple clinical assessments such as CBC, analysis of serum biochemistry and urine parameters, body weight and temperature, and heart and respiratory rates, none of which varied notably between blank- and penciclovir-treated cats. Importantly, no signs of renal, hepatic, gastrointestinal, or bone marrow toxicity were noted.

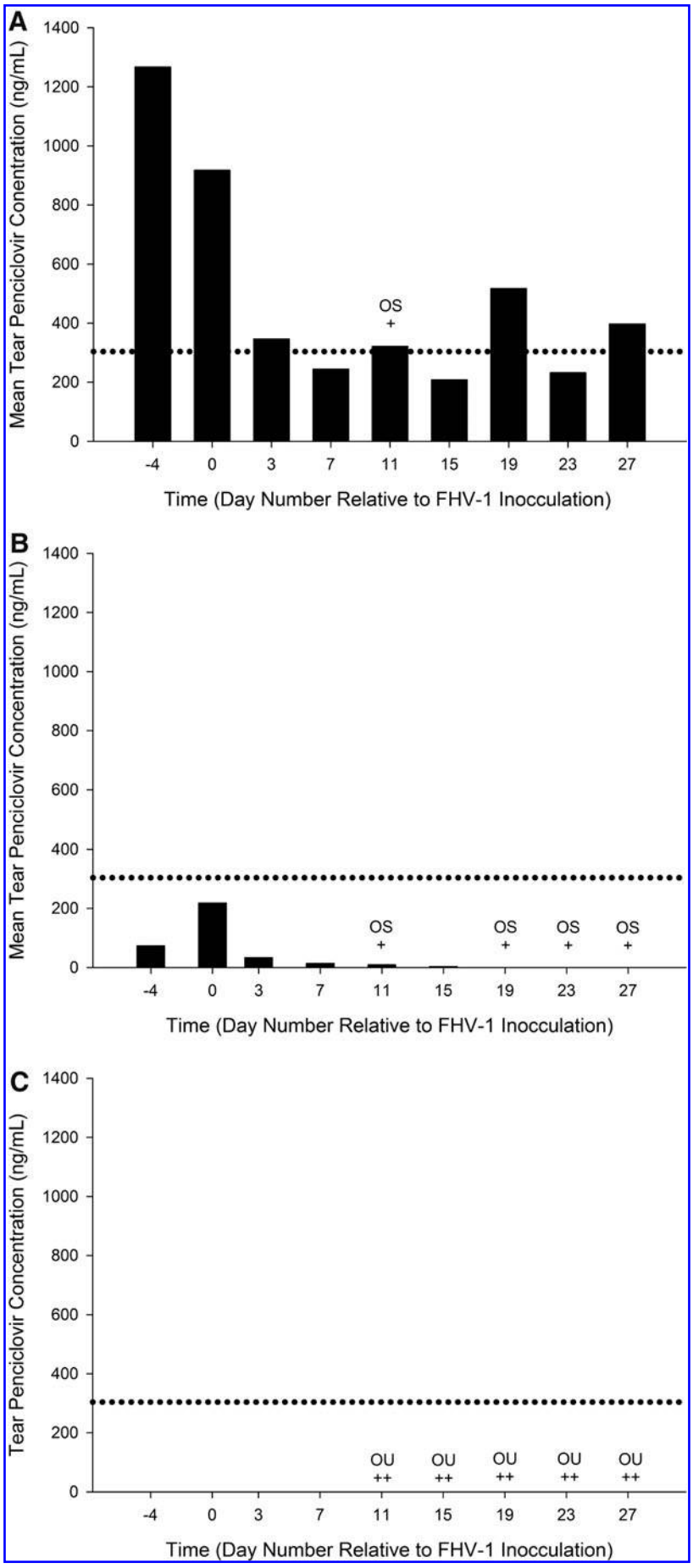

FIG. 7. Tear penciclovir concentration (*bars*; ng/mL) and presence of unilateral (+) or bilateral (++) corneal ulcers in 3 individual cats representative of those in which blank implants (4 cats) or penciclovir-containing implants (6 cats) were placed subconjunctivally on day Negative 8 or Negative 7 and removed on day 29. All cats were inoculated with FHV-1 on day 0. The lowest published IC_{50} of penciclovir for FHV-1 (ie, the ''target tear penciclovir concentration''),[39] is shown as a *horizontal dotted line*. **(A)** Corneal ulcers were detected infrequently in cats in which the target tear penciclovir concentration was reached. **(B)** Corneal ulcers were more frequent in cats in which the target tear penciclovir concentration was not achieved, or achieved inconsistently. **(C)** Corneal ulcers were most frequent and persistent in cats with a blank implant. OS, left eye; OU, both eyes.

This confirms earlier data suggesting that penciclovir (and its prodrug, famciclovir) appears to be very well tolerated by cats.[26–28,40,41,47] This is noteworthy because the related acyclic nucleoside—acyclovir—and its prodrug, valacyclovir, can be fatal in cats.[48,49] Weight loss in both groups of this study at the time of peak disease was similar to that reported for untreated cats infected with this inoculum,[26] and is unlikely to be attributable to the implant itself.

The lack of adverse systemic effects observed in cats receiving a subconjunctival penciclovir implant in this study is consistent with the low plasma penciclovir concentrations achieved (<10 ng/mL). However, these very low circulating plasma concentrations likely also explain the lack of notable antiviral effect noted in implanted cats. The lowest reported IC_{50} for penciclovir when tested *in vitro* with FHV-1 (304 ng/mL)[44] is at least 30-fold higher than the plasma penciclovir concentrations achieved in cats within this study. Furthermore, the plasma penciclovir concentrations achieved herein are about 100- to 200-fold lower than those achieved in cats successfully treated with orally administered famciclovir ($\sim$1,000–2,100 ng/mL).[26] By contrast, tear penciclovir concentrations from 5/6 treated cats in this study exceeded the targeted IC_{50}[44] on at least 1 occasion. Taken together, these data suggest that, with this implant, effective concentrations of penciclovir in the tears can be achieved in the absence of effective plasma concentrations, and that such local delivery methods might be very effective, especially in patients who cannot tolerate systemic antiviral drug delivery. The relative importance of plasma and tear penciclovir concentrations must also be assessed in light of the model used in this study. In this study, we used the established model of primary exposure to FHV-1. This enabled us to ensure uniformity of viral inoculum and clinical disease produced. Clearly, in a clinical setting, preemptive use of an implant in such patients would not be possible. Rather, we foresee that patients with chronic or recurrent herpetic ocular disease would be candidates for this mode of treatment. In those patients, it is expected that local (tear and ocular surface) concentrations of antiviral drugs would be of more relevance than circulating (plasma) concentrations. Based upon data from this pilot study, a trial would be of value in a larger number of patients with the diverse range of recurrent ocular surface disease seen with herpetic infections.

The relatively stable STT results recorded in this study suggest that tear production varied minimally over the study period. As a result, comparison of tear penciclovir concentrations across time points was possible. Tear penciclovir concentrations tended to be highest 1 week after implantation, suggesting a ''burst release'' from the implants that is consistent with previous *in vitro* studies of similar implants impregnated with acyclovir[33,50] or penciclovir.[32] However, it is possible that tear penciclovir concentrations in this study were also affected by conjunctival wound healing following implantation and initially, high tear penciclovir concentrations represented leakage through the surgical incision, with subsequent reductions due to wound closure and fibrosis. Following the initial period of high tear penciclovir concentrations, wide variations within and between subjects were noted, with values exceeding the targeted IC_{50}[44] on only 6 of 36 occasions, and in only 3 of 6 cats. Average daily tear penciclovir concentration exceeded IC_{50} in only 1 cat (in which it was 425 ng/mL/day). Further refinement of the implant biomaterial and its release pharmacokinetics so as to ensure more predictable release parameters appears necessary.

The marked variability in tear penciclovir concentration among cats permitted a fortuitous observation that warrants further study. Cats with higher tear penciclovir concentrations at virologically or clinically critical time points such as the time of inoculation or of peak clinical disease tended to have fewer corneal ulcers than did cats with inconsistent, low, or unrecordable tear penciclovir concentrations. When analyzed individually, cats in which tear penciclovir concentration exceeded IC_{50} more frequently tended to have lower viral loads, while those in which tear penciclovir concentrations were lower had more frequent corneal ulcers and higher viral loads, particularly at later time points. In addition, a sudden increase in viral load was noted in 1 cat within the blank-implanted group. This caused the average value of the entire group to be several logfold higher than that for the penciclovir-implanted group on day 28 (Fig. 3). Corneal ulcers observed in this particular blank-implanted cat were greater in frequency and severity than in any other cat from the blank- or penciclovir-implanted groups, with ulceration being bilateral on 5 consecutive observations in the latter part of the study. This observation further supports the hypothesis that ocular viral load and viral disease are likely to be better controlled with an implant that more consistently achieves at least IC_{50}.

These data are encouraging and support further refinement of the implant delivery system, or consideration of a well-tolerated, topical ophthalmic preparation of penciclovir. They are also similar to data from a study assessing oral administration of the penciclovir prodrug famciclovir,[26] where untreated cats had significantly higher ocular viral loads and worse disease. This finding is likely important given that viral shedding from infected cats represents the most important epidemiological source of infection of naive cats. Furthermore, our preliminary data suggest that higher tear penciclovir concentrations are associated with reduced corneal ulcer frequency and duration. This finding is important and novel because, while systemic famciclovir administration had dramatic positive effects in experimentally infected cats, it did not inhibit ulcer development.[26–28,39] Although this study did not prove that the relationship between tear penciclovir concentration and ulcer development was causal, it seems reasonable that this was due to penciclovir rather than the implant itself because notable differences in tear film parameters (STT, TFBUT, or GCD) were not detected between groups, and followed patterns seen previously.[26,39]

Conclusions

Subconjunctival placement of blank or penciclovir-impregnated implants was associated with increased aqueous tear production and mild conjunctivitis, but was generally well tolerated at the ocular surface and not associated with detectable systemic effect, or with substantial plasma penciclovir concentrations. Although tear penciclovir concentration ranged widely, tear penciclovir concentrations above or equal to the lowest published IC_{50}[44] were sometimes achieved, especially soon after implant placement, suggesting an initial burst release. Higher tear penciclovir concentrations were typically associated with fewer corneal

ulcers and lower viral loads. These pilot data suggest that a more reliable means of local delivery of penciclovir at or near the ocular surface may be useful in patients with herpetic disease, especially those who could not safely receive or do not require systemic therapy, including those experiencing ocular recrudescence without systemic signs, patients with hepatic, renal, or bone marrow dysfunction, and patients noncompliant for frequent topical dosing.

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Author Disclosure Statement

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