Title

Clinical and antiviral effect of a single oral dose of famciclovir administered to cats at intake to a shelter

Journal

The Veterinary Journal, 203(2)

ISSN

1090-0233

Authors

Litster, AL
Lohr, BR
Bukowy, RA
et al.

Publication Date

2015-02-01

DOI

10.1016/j.tvjl.2014.11.011

Peer reviewed

The Veterinary Journal 203 (2015) 199–204

Contents lists available at ScienceDirect

The Veterinary Journal

journal homepage: www.elsevier.com/locate/tvjl

Clinical and antiviral effect of a single oral dose of famciclovir administered to cats at intake to a shelter

A.L. Litster [a,*], B.R. Lohr [a], R.A. Bukowy [a], S.M. Thomasy [b], D.J. Maggs [b]

[a] Department of Veterinary Clinical Sciences, Purdue University, 625 Harrison Street, West Lafayette, IN 47907, USA
[b] Department of Surgical and Radiological Sciences, School of Veterinary Medicine, University of California-Davis, Davis, CA 95616, USA

ARTICLE INFO

Article history:
Accepted 20 November 2014

Keywords:
Cat
Feline herpesvirus
Famciclovir
Penciclovir
Shelter

ABSTRACT

Although famciclovir is efficacious in feline herpesvirus type 1 (FHV-1)-infected cats, effects of a single dose early in disease course have not been reported. In this two part, randomized, masked, placebo controlled study, cats received a single dose of 125 mg famciclovir ($n = 43$) or placebo ($n = 43$; pilot study), or 500 mg famciclovir ($n = 41$) or placebo ($n = 40$; clinical trial) on entering a shelter. FHV-1 PCR testing was performed, bodyweight and food intake were recorded, and signs of respiratory disease were scored prior to and 7 days following treatment. FHV-1 DNA was detected in 40% of cats in both parts at study entry. In the pilot study, ocular and nasal discharge scores increased from days 1 to 7 in famciclovir and placebo treated cats. Sneezing scores increased and bodyweight decreased in famciclovir-treated cats. The proportion of cats in which FHV-1 DNA was detected increased over time in all cats in the pilot study. In the clinical trial, food intake and median clinical disease scores for nasal discharge and sneezing increased from days 1 to 7 in both groups and demeanor scores worsened in famciclovir-treated cats. The proportion of cats shedding FHV-1 DNA was greater on day 7 than on day 1 in cats receiving 500 mg famciclovir. A single dose of famciclovir (125 or 500 mg) administered at shelter intake was not efficacious in a feline population in which 40% were already shedding FHV-1.

Introduction

Feline herpesvirus (FHV-1) is shed in oral, nasal and ocular secretions, and is transmitted by direct contact with infected cats, via sneezing, or through fomites and ineffective sanitation (Gould, 2011). At least 80% of infected cats remain persistently infected, since the virus becomes latent in neural tissue, especially within the trigeminal ganglia, as well as the optic nerves, olfactory bulbs and corneas (Reubel et al., 1993). This helps to account for its estimated seroprevalence of 50–97% in feline populations worldwide (Studdert and Martin, 1970; Ellis, 1981; Maggs et al., 1999; Bannasch and Foley, 2005; Byeong-Teck and Hee-Myung, 2008; Blanco et al., 2009).

Acutely infected cats and carrier cats undergoing viral reactivation shed FHV-1 (Gaskell and Povey, 1982) and display a variety of clinical signs, including depression, sneezing, conjunctival hyperemia and chemosis, corneal or conjunctival ulceration, nasal and ocular discharge and, less commonly, skin lesions (Persico et al., 2011). Viral shedding peaks at 7 days post-infection (Reubel et al., 1992) and lifelong latency occurs in approximately 80% of cats (Gaskell and Povey, 1977). Since the virus is reactivated by stress (Gaskell and Povey, 1982), and animal shelters are often stressful environments for cats (Bannasch and Foley, 2005), latent carriers are important reservoirs for disease transmission (Gaskell and Willoughby, 1999). Since viral shedding can occur without clinical signs (Gould, 2011), stress reduction through minimally invasive daily cage cleaning, providing hiding spaces and minimizing handling is vital (Bannasch and Foley, 2005). However, FHV-1 remains a major cause of respiratory and ocular disease in animal shelters; these conditions are common reasons for euthanasia in animal shelters (Bannasch and Foley, 2005).

Famciclovir was developed to increase bioavailability of its active metabolite, penciclovir, in humans (Pue et al., 1994). If administered to humans when symptoms commence, famciclovir can reduce the time to remission of clinical signs due to herpes simplex virus (HSV)-1 (Spruance et al., 2006) or HSV-2 (Aoki et al., 2006; Bodsworth et al, 2008). Two randomized, double-masked trials have investigated the effect of a single dose of famciclovir initiated by human patients at the onset of prodromal symptoms; both demonstrated efficacy for treatment of genital herpesvirus infections (Aoki et al., 2006; Spruance et al., 2006). Penciclovir has been used to treat disease due to HSV-1, HSV-2, varicella zoster virus and Epstein–Barr virus (Razonable, 2011).

In humans, famciclovir undergoes first pass deacetylation metabolism, predominantly in the blood, to produce the intermediate metabolite BRL 42359 (deoxypenciclovir; di-desacetyl famciclovir), which is readily converted to penciclovir by hepatic aldehyde oxidase. However, the precise mechanism responsible for conversion of

* Corresponding author. Tel.: +1 765 4183186.
E-mail address: catvet@purdue.edu (A.L. Litster).

http://dx.doi.org/10.1016/j.tvjl.2014.11.011
1090-0233/

famciclovir to penciclovir is not known (Thomasy et al., 2012b). In a human study, maximum plasma penciclovir concentrations (C_{max}) were achieved 30–45 min after administration of 125–750 mg famciclovir; neither time to C_{max} or half-life were dose-dependent (Pue et al., 1994).

Penciclovir has a relatively long intracellular half-life; 10 h in HSV-1 infected cells and 20 h in HSV-2 infected cells (Aoki et al., 2006). Penciclovir is converted to its active metabolite after three phosphorylation steps, the first being catalyzed by viral thymidine kinase in infected cells. The remaining two steps are catalyzed by cellular enzymes, resulting in relatively selective inhibition of viral DNA replication (Pue et al., 1994). In humans, the main route of penciclovir elimination following oral famciclovir administration is via the kidneys (Pue et al., 1994).

Compared to humans, cats absorb famciclovir relatively poorly after oral administration, and significant individual variation exists (Thomasy et al., 2012b). Furthermore, it is presumed that negligible feline hepatic aldehyde oxidase activity (Dick et al., 2005) is responsible for poor conversion of famciclovir to penciclovir (Maggs, 2010). In one feline study that assessed an oral dose of 15 mg/kg, the peak plasma penciclovir concentration (C_{max}) of 350 ± 180 ng/mL was detected at 4.6 ± 1.8 h, and the elimination half-life was 3.1 ± 0.9 h (Thomasy et al., 2007). In a later study by the same group, when the dose was increased 2.7-fold to 40 mg/kg, there was a fourfold increase in penciclovir C_{max}. However, a further 2.25-fold increase in dose from 40 mg/kg to 90 mg/kg did not result in a corresponding increase in plasma penciclovir C_{max} (Thomasy et al., 2012b).

In other studies, oral administration of famciclovir reduced clinical signs associated with FHV-1 infection (Malik et al., 2009) and decreased viral shedding (Thomasy et al., 2011). While tear concentrations of penciclovir in cats treated with 40 mg/kg famciclovir PO three times daily are likely to be effective against FHV-1 (Thomasy et al., 2012a), supplementary treatment with tear replacement products might be necessary due to alterations in the quality of tear film produced by reduced goblet cell density (Lim et al., 2009; Thomasy et al., 2011).

The aims of this randomized, placebo-controlled study were to determine whether a single oral dose of 125 mg or 500 mg of famciclovir, administered to cats at the time of shelter admission, would result in reduced clinical signs of upper respiratory tract disease (URTD) 7 days later, measured using a clinical scoring system, or reduced viral load at the pharyngeal and ocular surfaces, measured by quantitative PCR.

Materials and methods

Animals

One hundred and sixty seven adult cats at a large adoption-guarantee animal shelter in Chicago, Illinois (PAWS Chicago) were enrolled. All cats had been transferred from a large municipal shelter, where they had been admitted as strays or surrendered by their owners. Cat housing at the adoption-guarantee animal shelter consisted of a bank of nine solid-sided stainless steel cages all in one room. Each cage measured 60 cm × 60 cm × 60 cm (0.22 m^3), and the room contained only cats enrolled in the study. Cats were fed a mixture of commercially available canned and dry cat food (Hill's Science Diet Adult Optimal Care Original Cat Food) once daily. Dry food was fed in a quantity sufficient to be considered ad lib and a small additional amount of canned food was offered to every cat.

At intake (day 1), cats were examined by a shelter veterinarian and vaccinated subcutaneously with modified-live feline panleukopenia, FHV-1 and feline calicivirus (FCV) (Fel-O-Guard Plus 3; Boehringer Ingelheim Vetmedica) and inactivated rabies (Imrab 1; Merial) vaccines. Parasiticides were administered orally (pyrantel pamoate 5–10 mg/kg), topically (selamectin, Revolution, Pfizer) and SC (compounded praziquantel 6 mg/kg). Serology for feline immunodeficiency virus (FIV)/feline leukemia virus (FeLV) (SNAP Feline FeLV/FIV; IDEXX Laboratories) and Wood's lamp examination were performed on all cats on admission. Cats were excluded from enrollment if fluorescence was noted on Wood's lamp examination, circulating FIV antibodies or FeLV antigens were detected, or if they were estimated to be <6 months of age at the intake examination. Permission to perform this study was obtained from the Purdue Animal Care and Use Committee (approval number PACUC 10–126, date of approval 8 February 2012).

After enrollment but before oral administration of famciclovir or placebo on day 1, and then again on day 7, swab specimens (220115 BD Cultureswab; BD Diagnostics) were collected from both conjunctival sacs and the oropharynx. The swabs from each cat were combined and submitted for pooled analysis using real-time PCR identification of DNA/RNA from FHV-1, FCV, *Mycoplasma felis*, *Bordetella bronchiseptica* and *Chlamydia felis* (IDEXX FURD RealPCR).

Pilot study and clinical trial

In the pilot study, after intake procedures were completed and swab specimens were collected, cats were randomly allocated using a web-based randomization tool[1] to receive one orally administered tablet containing 125 mg famciclovir (Famvir 125 mg, Novartis; $n = 43$; median dose 32 mg/kg, range 16–52 mg/kg) or placebo (microcrystalline cell powder, cab-o-sil powder, stearic acid powder, corn starch powder, lactose 316 fast flow monohydrate, food color, green powder; Wedgewood Pharmacy; $n = 43$). The test drug or placebo was administered once only on day 1.

The pilot study was followed by a clinical trial, in which 81 different cats were randomly assigned to receive one orally administered tablet containing 500 mg famciclovir ($n = 41$; median dose 135 mg/kg, range 92–227 mg/kg) or the same placebo as the pilot study ($n = 40$) once on day 1 only. The first 27 cats enrolled in the clinical trial were randomly allocated to the famciclovir ($n = 14$) or placebo ($n = 13$) groups using a computer-generated randomization list[1] and were housed together (regardless of treatment group) in a room containing a bank of nine individual cages, as described for the pilot study. For the remainder of the clinical trial, cats in the famciclovir ($n = 27$) or placebo ($n = 27$) groups were housed in the same room, but all nine cats in the room were enrolled in the same treatment group. A coin toss was used to decide which treatment the initial group received, and treatment allocation was alternated thereafter. All trial medications were administered by a veterinary technician not responsible for subsequent scoring or analysis of outcomes. Hiding boxes (Feral cat and small mammal den, Heart of the Earth Animal Equipment) were provided in each cage for all cats enrolled in the clinical trial, but not for the pilot study.

Approximately 3 h after administration of trial medication, blood was collected from the first 35 cats enrolled in the clinical trial (famciclovir group: $n = 25$; placebo group: $n = 10$) for analysis of plasma BRL42359 and penciclovir concentrations. After collection into lithium heparin tubes (BD Vacutainer 6 mL plastic tubes), blood was centrifuged at room temperature (300 *g*/min for 10 min) before plasma was withdrawn in 0.5 mL aliquots using 1.5 mL pipettes (VWR Disposable Transfer Pipets, Graduated), placed into cryogenic tubes (VWR Neptune Cryogenic Tubes, 0.5 mL), and stored at –80 °C until subsequent penciclovir and BRL42359 analysis.

Evaluation of clinical signs of upper respiratory tract disease

One of two veterinarians masked to treatment allocation for the duration of the study graded clinical signs of ocular and URTD on day 1 (before trial medication was administered) and day 7 using a modified version of a four point scoring system (Litster et al., 2012; Table 1). Sneezing and coughing for each cat was recorded over a 30 min observation period. Food intake was estimated by observations of the amount of food consumed over the previous 24 h. Cats were weighed and body condition scores were recorded on days 1 and 7, and rectal temperature was recorded on day 1.

Analysis of plasma penciclovir and BRL42359

A stock solution (1 mg/mL) of BRL42359 (Novartis Animal Health) was prepared in methanol (Fisher Scientific) and stock solutions (1 mg/mL) of penciclovir (Novartis Animal Health) and acyclovir (Sigma Aldrich) were prepared in methanol (Fisher Scientific) and water (Burdick and Jackson); all solvents were of high performance liquid chromatography grade or better. Penciclovir and BRL42359 were combined into one working solution; working solutions were prepared by dilution of the 1 mg/mL stock solutions with methanol to form final concentrations of 0.0001, 0.001, 0.01 and 0.1 μg/μL. Plasma calibrators were prepared by dilution of the working standard solutions with drug-free plasma to final concentrations of 0.002, 0.01, 0.025, 0.1, 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 15, 20, 25, 30, 40, 50, 60 and 75 μg/mL. Penciclovir was quantified from 0.002 to 4 μg/mL and BRL42359 was quantified from 0.002 to 75 μg/mL. Calibration curves and negative control samples were prepared immediately prior to each quantitative assay. For additional verification of accuracy, quality control samples (plasma fortified with analyte at four concentrations within the standard curve) were included with each sample set.

Prior to analysis, 250 μL plasma was diluted with 250 μL acetonitrile:1 M acetic acid (9:1, V:V; Burdick and Jackson) containing 0.5 μg/mL acyclovir internal standard, to precipitate proteins. The samples were vortexed for 2 min, refrigerated for 20 min, vortexed on a GlasCol large capacity mixer for an additional 1 min, centrifuged in a Sorvall Super T21 at 3102 *g* for 10 min at 4 °C and 20 μL was injected into the liquid chromatography (LC)/mass spectrometry (MS) system.

[1] See: http://www.randomizer.org (accessed 1 December 2014).

Table 1
Clinical scoring system used in the pilot study and clinical trial.

	Score 0	Score 1	Score 2	Score 3
Ocular discharge	No ocular discharge	Small amount of serous discharge	Large amount of serous discharge	Mucopurulent discharge
Nasal discharge	No nasal discharge	Small amount of serous discharge	Large amount of serous discharge	Mucopurulent discharge
Respiration	Normal respiration; no sneezing	Mild difficulty breathing (mildly increased chest movements with no regular abdominal movements present during breathing)	Moderate difficulty breathing (increased chest movements with some regular abdominal movements present during breathing)	Severe difficulty breathing (increased chest movements with marked regular abdominal movements present during breathing)
Sneezing	No sneezing	Sneezes 1–2 times/30 min	Sneezes 3–4 times/30 min	Sneezes 5 or more times/30 min
Coughing	No coughing	Coughs 1–2 times/30 min	Coughs 3–4 times/30 min	Coughs 5 or more times/30 min
Demeanor	Bright, alert, reactive	Quiet, lethargic	Depressed but responds to human contact	Severely depressed, demeanor does not change in response to human contact
Food intake	Eating normally	Mildly reduced appetite	Moderately reduced appetite	Anorexic
Oral pathology	No inflammation present	Mild or moderate inflammation present; gums do not bleed when touched	Moderate inflammation present; gingiva bleed when touched	Severe inflammation present; gums bleed when touched

Plasma penciclovir and BRL42359 concentrations were measured by LC tandem-MS using positive heated electrospray ionization at 300 °C. Quantitative analysis of plasma was performed on a TSQ Vantage triple quadrupole mass spectrometer (Thermo Scientific) coupled with a 1100 series liquid chromatography system (Agilent Technologies) and operated in laminar flow mode. The spray voltage was 3500 V, the vaporizer temperature was 292 °C and the sheath and auxiliary gas were 40 and 40 arbitrary units, respectively. Product masses and collision energies of each analyte were optimized by infusing the internal standards into the mass spectrometer. Chromatography employed an Eclipse-XDB-Phenyl column (2.1 mm × 150 mm, particle size 5 μm; Agilent Technologies) and a linear gradient of acetonitrile in water with a constant 0.2% formic acid (Alfa Aesar; 97% formic acid) at a flow rate of 0.50 mL/min. The initial acetonitrile concentration was held at 0% for 0.5 min, increased to 90% over 6 min, and held at that concentration for 0.17 min, before re-equilibrating for 3.33 min at initial conditions.

Detection and quantification were conducted using selected reaction monitoring of the initial precursor ions for all analytes: penciclovir (mass to charge ratio 254.1 m/z), BRL42359 (mass to charge ratio 238.2 m/z) and the internal standard acyclovir (mass to charge ratio 226.1 m/z). Response for the product ion for penciclovir (110.0, 135.1, 152.1 m/z), BRL42359 (109.1, 119.1, 136.1 m/z) and the internal standard (acyclovir 110.1, 135.1, 152.1 m/z) were plotted, and peaks at the proper retention time were integrated using Quanbrowser software (Thermo Scientific). Quanbrowser software also was used to generate calibration curves and quantify the analytes in all samples by regression analysis. A weighting factor of 1/X was used for all calibration curves. All calibration curves had correlation coefficients (r^2) of at least 0.99. The limit of quantitation was 0.002 μg/mL for penciclovir and BRL42359.

Data analysis

Proportions of cats in each group of each gender and of each neuter status were compared using χ^2 tests. Scores were compared within each group (day 1 vs. day 7) using the Wilcoxon matched-pairs signed rank test, whereas comparisons between groups on each day were made using the Mann–Whitney test. The proportion of cats from which FHV-1 DNA was detected by PCR was compared between groups and between days using the χ^2 or Fisher's exact test. To permit analysis of the effect of viral load on treatment outcomes, semi-quantitative PCR data were used to assign cats in the clinical trial to one of three categories: low (<38,000 FHV-1 viral particles/swab), intermediate (38,000–150,000 viral particles/swab) or high (>150,000 viral particles/swab) viral load. Fisher's exact test was used to compare the number of cats within each viral load category with the number of cats within the remaining two categories. Least squares linear regression was used to evaluate relationships between famciclovir dose and plasma BRL42359 concentration, and between famciclovir dose and plasma penciclovir concentration. SigmaPlot 12.0 was used for least squares regression and GraphPad Prism 5.0 for other analyses; P values < 0.05 were considered to be significant.

Results

Pilot study

Eighty-six cats were enrolled in the pilot study, with 43 in the famciclovir group (10 males, M, 12 neutered males, MN, 19 females, F, and 2 female spayed, FS), and 43 in the placebo group (16 M, 10 MN, 15 F, 2 FS). There were no differences between groups in the proportions of M vs. F, disregarding neuter status ($P = 0.4$), or in neuter status, disregarding sex ($P = 0.6$).

Clinical data and statistical comparisons for the pilot study are shown in Table 2. Median clinical disease scores did not differ between treatment groups at days 1 or 7. Median clinical disease scores increased from days 1 to 7 in both groups for ocular and nasal discharge and also for sneezing in the placebo group. Bodyweight decreased significantly over the study period in the famciclovir group ($P < 0.01$), but not in the placebo group ($P = 0.08$). On day 1, FHV-1 DNA was detected in the conjunctival sac and/or oropharynx of

Table 2
Median (interquartile range) clinical data and statistical comparisons from the pilot study in which cats received 125 mg famciclovir ($n = 43$) or placebo ($n = 43$) orally once on day 1.

Parameter	Clinical data				Statistical comparisons (P values)			
	Day 1		Day 7		Day 1	Day 7	Famciclovir	Placebo
	Famciclovir	Placebo	Famciclovir	Placebo	Famciclovir vs. placebo	Famciclovir vs. placebo	Day 1 vs. day 7	Day 1 vs. day 7
Bodyweight (kg)	3.9 (3.4–4.6)	3.8 (3.0–4.7)	3.6 (3.1–4.5)	3.7 (3.1–4.4)	0.6	1.0	0.005	0.088
Age (months)	24 (12–36)	24 (12–36)	NR	NR	0.6	NR	NR	NR
Rectal temperature (°C)	38.3 (38.3–38.9)	38.3 (38.3–38.9)	NR	NR	0.3	NR	NR	NR
Ocular discharge	0 (0–1)	0 (0–1)	1 (0–1)	1 (0–1)	0.8	0.5	0.001[a]	0.01[a]
Nasal discharge	0 (0–0)	0 (0–0)	0 (0–1)	0 (0–1)	0.05	0.8	0.0001[a]	0.005[a]
Respiration	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	1.0	0.6	0.2	0.3
Sneezing	0 (0–0)	0 (0–0)	0 (0–1)	0 (0–2)	0.2	0.6	0.002[a]	0.07
Coughing	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	1.0	0.3	1.0	1.0
Demeanor	1 (0–1)	1 (0–1)	0 (0–1)	0 (0–1)	0.7	0.5	0.9	0.2
Food intake	2 (1–3)	2 (0.75–2)	1 (1–2)	1 (1–2)	0.5	0.7	0.2	0.4

[a] Within group scores were statistically higher on day 7 than on day 1 ($P < 0.05$).
NR, not recorded.

Table 3
Median (interquartile range) clinical data and statistical comparisons from the clinical trial in which cats received 500 mg famciclovir ($n = 41$) or placebo ($n = 40$) orally once on day 1.

Parameter assessed	Clinical data				Statistical comparisons (P values)			
	Day 1		Day 7		Day 1	Day 7	Famciclovir	Placebo
	Famciclovir	Placebo	Famciclovir	Placebo	Famciclovir vs. placebo	Famciclovir vs. placebo	Day 1 vs. day 7	Day 1 vs. day 7
Bodyweight (kg)	3.7 (3.3–4.4)	3.7 (3.2–4.4)	3.9 (3.4–4.4)	4.0 (3.1–4.4)	0.9135	0.8985	0.0322	0.1845
Age (months)	12 (12–24)	12 (11–36)	NR	NR	0.6321	NR	NR	NR
Rectal temperature (°C)	38.3 (38.3–38.9)	38.3 (37.8–38.9)	38.9 (38.3–39.4)	38.3 (38.3–38.9)	0.9623	0.0344	0.1230	0.3606
Ocular discharge	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0.0403[a]	0.3284	0.0890	0.5877
Nasal discharge	0 (0–0)	0 (0–0)	0 (0–1)	0 (0–0.75)	0.9860	0.4547	0.0023[b]	0.0151[b]
Respiration	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	1.0000	0.9834	0.1489	0.1489
Sneezing	0 (0–0)	0 (0–0)	0 (0–2)	0 (0–1)	0.9900	0.4666	0.0005[b]	0.0004[b]
Coughing	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	1.0000	0.6033	0.3458	1.0000
Demeanor	0 (0–0)	0 (0–1)	0 (0–0)	0 (0–0)	0.3233	0.0615	0.0016[b]	0.4099
Food intake	0 (0–0)	0 (0–1)	0 (0–0)	0 (0–1)	0.6424	0.9399	0.0065[b]	0.0208[b]

[a] Ocular discharge score was statistically higher on day 1 in the placebo group than in the famciclovir group ($P < 0.05$).
[b] Within group scores were statistically higher on day 7 than on day 1 ($P < 0.05$).
NR, not reported.

34/86 (39.5%) cats, including 17/43 (39.5%) cats in the famciclovir group and 17/43 (39.5%) cats in the placebo group ($P = 1.0$). On day 7, FHV-1 DNA was detected in 73/86 (84.9%) cats, including 37/43 (86%) in the famciclovir group and 36/43 (83.7%) in the placebo group ($P = 0.8$). Considering all cats in both treatment groups, the proportion of cats in which FHV-1 DNA was detected was significantly greater on day 7 (73/86, 84.9%) than on day 1 (34/86, 39.5%; $P < 0.01$); this significant increase in FHV-1 shedding over time was noted in cats treated with famciclovir ($P < 0.01$) and in the placebo group ($P < 0.01$).

Clinical trial

Eighty-one cats were enrolled in the clinical trial, including 41 in the famciclovir group (23 M, 5 MN, 10 F, 3 FS) and 40 in the placebo group (17 M, 6 MN, 13 F, 4 FS). There were no differences between groups in the proportions of M vs. F, disregarding neuter status ($P = 0.3$), or in neuter status, disregarding sex ($P = 0.6$). Clinical data and statistical comparisons for the pilot study are shown in Table 3. Median clinical disease scores did not vary between treatment groups at days 1 or 7. Median clinical disease scores increased from days 1 to 7 in both groups for nasal discharge, sneezing and food intake, whereas demeanor scores worsened in the famciclovir group.

On day 1, FHV-1 DNA was detected in the conjunctival fornix or oropharynx of 33/81 (40.7%) cats, including 15/41 (36.6%) in the famciclovir group and 18/40 (45.0%) in the placebo group ($P = 0.4$). On day 7, FHV-1 DNA was detected in 61/81 (75.3%) cats; there were significantly more in the famciclovir group (36/41; 87.0%) than in the placebo group (25/40; 62.5%; $P < 0.01$). The proportion of all cats regardless of treatment group and in which FHV-1 DNA was detected was significantly greater on day 7 (61/81, 75.3%) than on day 1 (33/81, 40.7%; $P < 0.01$). This increase in FHV-1 shedding over time was significant in cats treated with famciclovir ($P < 0.01$), but not in the placebo group ($P = 0.1$). Viral loads (low, intermediate or high) in the famciclovir group and the placebo group are shown on day 1 in Fig. 1A and day 7 in Fig. 1B.

Plasma penciclovir and BRL 42359 concentrations

In the famciclovir group ($n = 35$), median (range) plasma penciclovir and BRL42359 concentrations were 2.3 (1.3–5.1) μg/mL and 46.5 (27.4–82.6) μg/mL, respectively, following a median (range) dose of 132 (91–227) mg of famciclovir/kg. In the placebo group ($n = 10$), median (range) plasma penciclovir and BRL42359 concentrations were 0.003 (0–0.006) μg/mL and 0.012 (0–0.028) μg/mL, respectively. In the famciclovir group, the plasma BRL 42359 concentration was significantly correlated with famciclovir dose ($P < 0.01$; Fig. 2A). There was no significant correlation between famciclovir dose and plasma penciclovir concentration in the famciclovir group ($P = 0.8$; Fig. 2B).

Discussion

In this study, treatment with a single oral dose of 125 or 500 mg famciclovir once at the time of intake of cats into a shelter did not limit development of signs of URTD or reduce FHV-1 shedding in

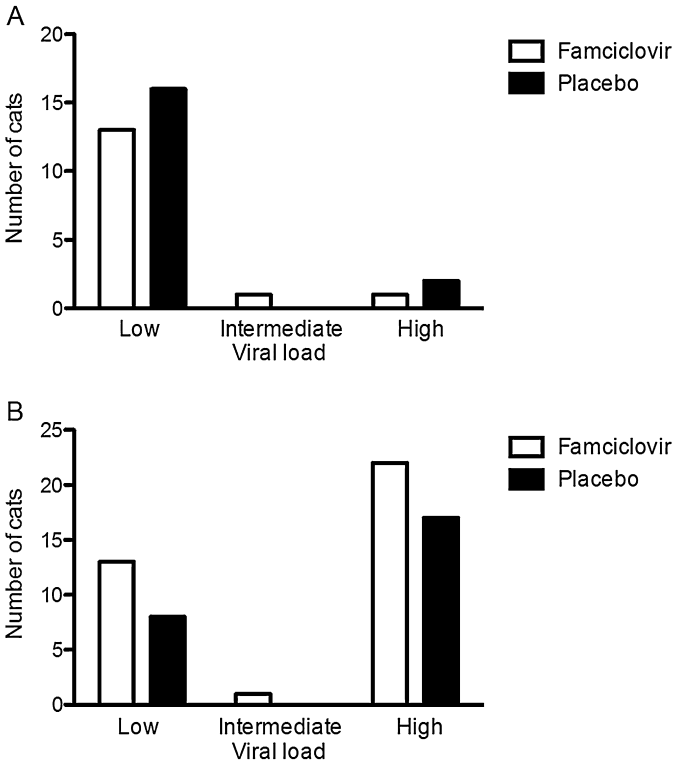

Fig. 1. Viral loads (low, intermediate or high) on days 1 (A) and 7 (B) in cats receiving either 500 mg famciclovir or placebo once immediately following PCR on day 1.

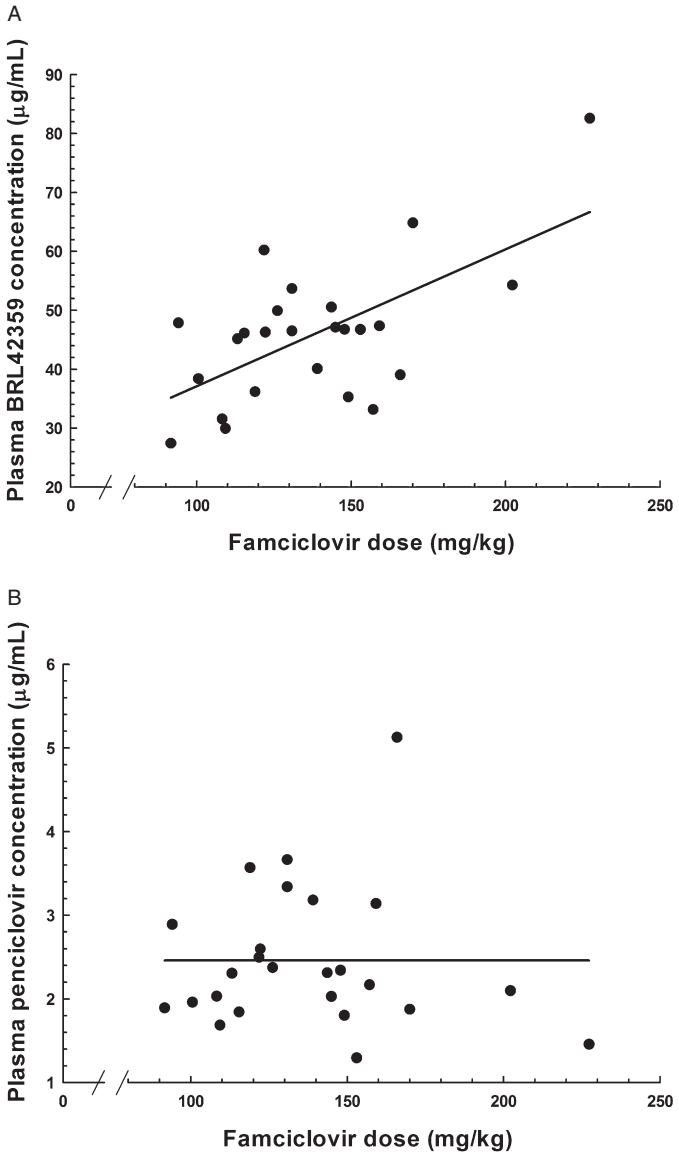

Fig. 2. Correlation between famciclovir dose and plasma BRL42359 or penciclovir concentrations in 25 cats sampled approximately 3 h following a single oral dose of 500 mg famciclovir. (A) Plasma BRL 42359 concentrations were significantly correlated with famciclovir dose ($P < 0.001$). (B) There was no significant correlation between plasma penciclovir concentration and famciclovir dose ($P = 0.79$).

medicated cats. In cats administered 125 mg famciclovir, corresponding to a median (range) dose of 32 (16–52) mg/kg, once only at study entry, nasal and ocular discharge, and sneezing scores, all increased significantly over the 7 day study period. Similarly, cats administered 500 mg famciclovir, corresponding to a median (range) dose of 135 (92–227) mg/kg, once only at study entry demonstrated significant deterioration in food intake, as well as nasal discharge, sneezing and demeanor scores, and increased viral shedding over the 7 day study period. Importantly, the increased viral shedding and decreased demeanor scores in cats receiving 500 mg famciclovir, and the decreased sneezing score in cats receiving 125 mg famciclovir, were not seen in placebo-treated cats. Given that all cats in the famciclovir-treated groups had detectable plasma penciclovir concentrations, it appears that cats received the medication as expected.

Furthermore, the median (range) maximum plasma penciclovir concentrations of 2.3 (2.3–5.1) µg/mL achieved in cats at the approximate time of maximum plasma concentrations (Thomasy et al., 2011) in the present clinical trial are equivalent to the C_{max} proven to be effective in reducing clinical signs and viral shedding in cats experimentally inoculated with FHV-1 (2.0 µg/mL; Thomasy et al., 2011). Therefore, it is likely that the lack of efficacy seen in the present study is due to the short duration of therapy or the timing of therapy relative to viral reactivation rather than the dose given. Cats in the present study were transferred to this shelter from another shelter and about 40% of cats in the treatment and placebo groups of both arms of the study were already shedding FHV-1 prior to administration of famciclovir or placebo. Therefore, it is likely that viral reactivation had already occurred in these cats at the time of intake at the shelter where the study was conducted and that administration of famciclovir was therapeutic rather than prophylactic.

If viral reactivation had not yet occurred on day 1 of the study, it is possible that a single dose of famciclovir might have been more efficacious, as was the case when used as a prophylactic drug in humans with genital herpesvirus infections (Aoki et al., 2006; Spruance et al., 2006). Doses in the current trial were selected for their potential economic and logistic advantages. When the pilot study demonstrated that 125 mg/cat was ineffective, a dose of 500 mg/cat was selected for the clinical trial, as it was likely to be affordable in many shelters.

In the clinical trial, a single oral dose of famciclovir (500 mg) administered to 25 cats resulted in plasma penciclovir concentrations approximately 3 h following administration that were previously demonstrated to be efficacious (Thomasy et al., 2011). In contrast, plasma penciclovir concentrations obtained from 10 cats approximately 3 h after administration of a placebo were near the limit of detection and were unlikely to have clinical relevance. A significant correlation was observed between dose of famciclovir and and plasma concentration of the intermediate metabolite, BRL42359, while no correlation was observed between famciclovir dose and plasma concentration of penciclovir. This observation is consistent with studies by Thomasy et al. (2007, 2012b) suggesting that conversion of BRL42359 to penciclovir is saturable and nonlinear in cats. These results suggest that increasing the famciclovir dose further would be unlikely to alter the lack of efficacy observed in the present study.

This study had a number of limitations. Although the scorers were masked to treatment allocation for the pilot study and the clinical trial, they were aware of the change in methodology between the pilot study and the clinical trial. In the clinical trial, cats allocated to a single (masked) treatment group were housed together for the 7 day study period, perhaps introducing an opportunity for bias. Secondly, FHV-1 PCR results may be positive (1) for up to 15 days after vaccination of cats, even in the absence of clinical signs; (2) for up to 25 days in cats induced to shed virus under experimental stress; and (3) for a minimum of 4 weeks in experimentally infected cats (Weigler et al., 1997).

Additionally, PCR is unable to discriminate between vaccine virus, field strain virus and latent virus, and it cannot differentiate between live virus and viral fragments remaining after clinical resolution has occurred (Maggs and Clarke, 2005). While swabs with positive PCR results collected at enrollment in our study were unlikely to be from vaccine virus, it is possible that the day 7 results were affected by recent vaccination. However, a recent shelter-based study of 543 cats reported that, while the prevalence of FHV-1 determined by PCR was higher in cats housed for short periods in shelters, PCR results were not affected by recent vaccination (McManus et al., 2014). Another study of FHV-1-naïve kittens vaccinated against FHV-1 using either intranasal or subcutaneous vaccine demonstrated that FHV-1 DNA was amplified by PCR only after intranasal vaccination; none of the subcutaneously vaccinated kittens had detectable viral DNA after vaccination (Reagan et al., 2014).

Additionally, differences in stage of incubation or disease course in cats at the time of enrollment could have affected the comparison of PCR results in each cat from days 1 and 7. Since we did not have access to the medical history of any of the cats, it is not possible to state whether they were cats undergoing primary infection, chronic infection, or recent reactivation. It is possible that relatively small group sizes and a clinical scoring system limited to only four possible scores reduced the statistical power of the study, thereby hampering our ability to demonstrate a difference between treatment groups. However, a more discriminatory scoring system might have been predisposed to increased inter-observer variability, limiting its accuracy and utility.

Conclusions

Control of FHV-1 infection in shelter-housed cats should be based on appropriate housing designed to reduce stress, a vaccination protocol appropriate to the shelter, thorough cleaning and disinfection, and the isolation and treatment of clinically affected cats. Under the conditions tested in our randomized, masked, placebo-controlled study, a single oral dose of 125 or 500 mg of famciclovir administered at shelter intake was not efficacious in cats of which approximately 40% were already shedding virus at the time therapy was instituted.

Conflict of interest statement

Dr Maggs has received consulting honoraria from Novartis Animal Health. Dr Thomasy has received honoraria for a speaking engagement for Novartis Animal Health. None of the other authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

Acknowledgements

This study was supported, in part, by a grant from the Bernice Barbour Foundation (Grant Number: 09472013) and Maddie's Fund (Grant Number: 205394). The Purdue Maddie's Shelter Medicine Program is underwritten by a grant from Maddie's Fund(r), The Pet Rescue Foundation (<http://www.maddiesfund.org>), helping to fund the creation of a no-kill nation. We thank PAWS Chicago, Dr Jessica Von Waldau, Dr Barbara Kompare, Kristen Hall and Lee DiVita for their assistance. We are also grateful to Dr Christian Leutenegger and Marko Estrada, IDEXX Laboratories, West Sacramento, CA, USA, for assistance with diagnostic testing.

References

Aoki, F.Y., Tyring, S., Diaz-Mitoma, F., Gross, G., Hamed, K., 2006. Single-day, patient-initiated famciclovir therapy for recurrent genital herpes: A randomized, double-blind, placebo-controlled trial. Clinical Infectious Diseases 42, 8–13.

Bannasch, M.J., Foley, J.E., 2005. Epidemiologic evaluation of multiple respiratory pathogens in cats in animal shelters. Journal of Feline Medicine and Surgery 7, 109–119.

Blanco, K., Prendas, J., Cortes, R., Jimenez, C., Dolz, G., 2009. Seroprevalence of viral infections in domestic cats in Costa Rica. Journal of Veterinary Medical Science 5, 661–663.

Bodsworth, N., Bloch, M., McNulty, A., Denham, I., Doong, N., Trottier, S., Adena, M., Bonney, M., Agnew, J., Australo-Canadian FaST Famciclovir Short-Course Herpes Therapy Study Group, 2008. 2-Day versus 5-day famciclovir as treatment of recurrences of genital herpes: Results of the FAST study. Sexual Health 5, 219–225.

Byeong-Teck, K., Hee-Myung, P., 2008. Prevalence of feline herpesvirus 1, feline calicivirus and *Chlamydophila felis* in clinically normal cats at a Korean animal shelter. Journal of Veterinary Science 9, 207–209.

Dick, R.A., Kanne, D.B., Casida, J.E., 2005. Identification of aldehyde oxidase as the neonicotinoid nitroreductase. Chemical Research in Toxicology 18, 317–323.

Ellis, T.M., 1981. Feline respiratory virus carriers in clinically healthy cats. Australian Veterinary Journal 57, 115–118.

Gaskell, R., Willoughby, K., 1999. Herpesviruses of carnivores. Veterinary Microbiology 69, 73–88.

Gaskell, R.M., Povey, R.C., 1977. Experimental induction of feline viral rhinotracheitis virus re-excretion in FVR recovered cats. Veterinary Record 100, 128–133.

Gaskell, R.M., Povey, R.C., 1982. Transmission of feline viral rhinotracheitis. Veterinary Record 111, 359–362.

Gould, D., 2011. Feline herpesvirus-1 ocular manifestations, diagnosis and treatment options. Journal of Feline Medicine and Surgery 13, 333–346.

Lim, C.C., Reilly, C.M., Thomasy, S.M., Kass, P.H., Maggs, D.J., 2009. Effects of feline herpesvirus type 1 on tear film break up time, Schirmer tear test results, and conjunctival goblet cell density in experimentally infected cats. American Journal of Veterinary Research 70, 394–403.

Litster, A.L., Constable, P., Wu, C.C., 2012. Comparison of the efficacy of amoxicillin/clavulanic acid, cefovecin, and doxycycline in the treatment of upper respiratory tract disease in cats housed in an animal shelter. Journal of the American Veterinary Medical Association 241, 218–226.

Maggs, D.J., 2010. Antiviral therapy for feline herpesvirus infections. Veterinary Clinics of North America. Small Animal Practice 40, 1055–1062.

Maggs, D.J., Clarke, H.E., 2005. Relative sensitivity of polymerase chain reaction assays used for detection of feline herpesvirus type 1 DNA in clinical samples and commercial vaccines. American Journal of Veterinary Research 66, 1550–1555.

Maggs, D.J., Lappin, M.R., Reif, J.S., Collins, J.K., Carman, J., Dawson, D.A., Bruns, C., 1999. Evaluation of serologic and viral detection methods for diagnosing feline herpesvirus-1 infection in cats with acute respiratory tract or chronic ocular disease. Journal of the American Veterinary Medical Association 214, 502–507.

Malik, R., Lessels, N.S., Webb, S., Meek, M., Graham, P.G., Vitale, C., Norris, J.M., Power, H., 2009. Treatment of feline herpesvirus-1 associated disease in cats with famciclovir and related drugs. Journal of Feline Medicine and Surgery 1, 40–48.

McManus, C.M., Levy, J.K., Andersen, L.A., McGorray, S.P., Leutenegger, C.M., Gray, L.K., Hilligas, J., Tucker, S.J., 2014. Prevalence of upper respiratory pathogens in four management models for unowned cats in the South-East United States. The Veterinary Journal 201, 196–201.

Persico, P., Roccabianca, P., Corona, A., Vercelli, A., Cornegliana, L., 2011. Detection of feline herpes virus 1 via polymerase chain reaction and immunohistochemistry in cats with ulcerative facial dermatitis, eosinophilic granuloma complex reaction patterns and mosquito bite hypersensitivity. Veterinary Dermatology 22, 521–527.

Pue, M.A., Pratt, S.K., Fairless, A.J., Fowles, S., Laroche, J., Georgiou, P., Prince, W., 1994. Linear pharmacokinetics of penciclovir following administration of single oral doses of famciclovir 125, 250, 500 and 750 mg to healthy volunteers. Journal of Antimicrobial Chemotherapy 33, 119–127.

Razonable, R.R., 2011. Antiviral drugs for viruses other than human immunodeficiency virus. Mayo Clinic Proceedings 86, 1009–1026.

Reagan, K.L., Hawley, J.R., Lappin, M.R., 2014. Concurrent administration of an intranasal vaccine containing feline herpesvirus-1 (FHV-1) with a parenteral vaccine containing FHV-1 is superior to parenteral vaccination alone in an acute FHV-1 challenge model. The Veterinary Journal 201, 202–206.

Reubel, G.H., George, J.W., Barlough, J.E., Higgins, J., Grant, C.K., Pedersen, N.C., 1992. Interaction of acute feline herpesvirus-1 and chronic feline immunodeficiency virus infections in experimentally infected specific pathogen free cats. Veterinary Immunology and Immunopathology 35, 95–119.

Reubel, G.H., Ramos, R.A., Hickman, M.A., Rimstad, E., Hoffmann, D.E., Pedersen, N.C., 1993. Detection of active and latent feline herpesvirus 1 infections using the polymerase chain reaction. Archives of Virology 132, 409–420.

Spruance, S.L., Bodsworth, N., Resnick, H., Conant, M., Oeuvray, C., Gao, J., Hamed, K., 2006. Single-dose, patient-initiated famciclovir: A randomized, double-blind, placebo-controlled trial for episodic treatment of herpes labialis. Journal of the American Academy of Dermatology 55, 47–53.

Studdert, M.J., Martin, M.C., 1970. Virus diseases of the respiratory tract of cats. 1. Isolation of feline rhinotracheitis virus. Australian Veterinary Journal 46, 99–104.

Thomasy, S.M., Maggs, D.J., Moulin, N.K., Stanley, S.D., 2007. Pharmacokinetics and safety of penciclovir following oral administration of famciclovir to cats. American Journal of Veterinary Research 11, 1252–1258.

Thomasy, S.M., Lim, C.C., Reilly, C.M., Kass, P.H., Lappin, M.R., Maggs, D.J., 2011. Evaluation of orally administered famciclovir in cats experimentally infected with feline herpesvirus type-1. American Journal of Veterinary Research 1, 85–95.

Thomasy, S.M., Covert, J.C., Stanley, S.D., Maggs, D.J., 2012a. Pharmacokinetics of famciclovir and penciclovir in tears following oral administration of famciclovir to cats: A pilot study. Veterinary Ophthalmology 5, 299–306.

Thomasy, S.M., Whittem, T., Bales, J.L., Ferrone, M., Stanley, S.D., Maggs, D.J., 2012b. Pharmacokinetics of penciclovir in healthy cats following oral administration of famciclovir or intravenous infusion of penciclovir. American Journal of Veterinary Research 73, 1092–1099.

Weigler, B.J., Guy, J.S., Nasisse, M.P., Hancock, S.I., Sherry, B., 1997. Effect of a live attenuated intranasal vaccine on latency and shedding of feline herpesvirus 1 in domestic cats. Archives of Virology 142, 2389–2400.