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

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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

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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).