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REVIEW

A review of antiviral drugs and other compounds with activity against feline herpesvirus type 1

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Abstract

Feline herpesvirus type 1 (FHV-1) is a common and important cause of ocular surface disease, dermatitis, respiratory disease, and potentially intraocular disease in cats. Many antiviral drugs developed for the treatment of humans infected with herpesviruses have been used to treat cats infected with FHV-1. Translational use of drugs in this manner ideally requires methodical investigation of their *in vitro* efficacy against FHV-1 followed by pharmacokinetic and safety trials in normal cats. Subsequently, placebo-controlled efficacy studies in experimentally inoculated animals should be performed followed, finally, by carefully designed and monitored clinical trials in client-owned animals. This review is intended to provide a concise overview of the available literature regarding the efficacy of antiviral drugs and other compounds with proven or putative activity against FHV-1, as well as a discussion of their safety in cats.

Key Words: antiviral therapy, feline herpesvirus, interferon, lysine, nucleoside analogues, virology

INTRODUCTION

Feline herpesvirus type 1 (FHV-1) is a common and important cause of ocular surface disease, dermatitis, respiratory disease, and potentially intraocular disease in cats.¹ However, an increasing array of drugs with antiviral efficacy against FHV-1 and an improved understanding of their mechanisms of actions, indications, and limitations have led to critical improvements in veterinarians' ability to control feline herpetic syndromes. In a 1995 report of 14 client-owned cats with herpetic ocular disease treated with topically applied trifluridine, idoxuridine, or vidarabine, 43% failed to improve or worsened.² More recently, a similar review described 59 client-owned cats with ocular disease attributed to FHV-1 and treated orally with famciclovir.³ Clinical improvement was noted by the treating veterinarian in 85% of cats and by their owners in 93% of cats. Clearly, antiviral therapy for FHV-1 has come a long way in 20 years. The present article is intended to provide a concise review of the available literature regarding the efficacy of antiviral drugs and other compounds with proven or putative activity against FHV-1, as well as a discussion of their safety in cats.

ANTIVIRAL DRUGS

Conceivably, antiviral drugs could target any step in the viral replicative process from viral adsorption to release from the host cell. To date, however, most effective antiviral therapies target viral proteins responsible for DNA synthesis,^{4,5} and their safety depends, in large part, on how virus-specific that disruption of DNA is. Therefore, while most antiviral drugs have some efficacy against FHV-1, their safety in cats is not readily predicted from their behavior in other hosts, and their efficacy against FHV-1 is not predicted from their efficacy against other viruses – even the closely related human herpes simplex virus type 1 (HSV-1; Table 1). In addition, there are no drugs currently approved in the USA for treatment of herpetic disease in cats. These basic virologic concepts can be used to guide prescribing of antiviral drugs in general (Box 1).

Whenever a drug developed for treatment of humans infected with a herpesvirus is used to treat a cat infected with FHV-1, 2 major assumptions must be made—that the drug is efficacious against FHV-1 and that it is safe in cats. For these reasons, methodical investigation of *in vitro*

Table 1. Efficacy of various antiviral drugs against feline herpesvirus and herpes simplex virus type 1. Efficacy is reported as median (range) concentration (μM) at which *in vitro* viral replication is inhibited by 50% (IC_{50}), therefore a *lower* IC_{50} equates to *greater* efficacy. Drugs are ranked (left to right) in order of decreasing efficacy against feline herpesvirus. Note the different ranking for herpes simplex virus type 1.

	IC_{50} (μM)											
	HPMPA	IDU	GCV	PCV	PMEDAP	BDVU	TFU	CDV	VDB	ADV	ACV	PFA
FHV-1	0.23	5.6 (4.3–6.8)	8.9 (5.2–13)	14 (1.2–130)	14	18 (5.1–30.1)	19 (0.67–1350)	19 (7.9–168)	21	73	150 (16–111000)	187 (140–230)
HSV-1	22	2.1	0.39	1.5	6.9	0.3	0.5	19	30	21	0.8	68
References	31, 97	25, 58, 98	25, 59, 99	25, 29, 31, 43, 44, 11	59, 101	31, 58, 102	31, 33, 58, 103	25, 29, 44, 59, 104, 105	58, 103	59, 106	25, 29, 31, 33, 44, 59, 102, 107	25, 59, 102

ACV, Acyclovir; ADV, Adefovir; BDVU, Bromovinyldeoxyuridine; CDV, Cidofovir; FHV-1, feline herpesvirus type 1; GCV, Ganciclovir; HPMPA, (S)-9-(3-hydroxy-2-phosphonylmethoxypropyl) adenine; HSV-1, herpes simplex virus type 1; IDU, Idoxuridine; PCV, Penciclovir; PFA, Foscarnet; PMEDAP, 9-(2-phosphonylmethoxyethyl)-2, 6-diaminopurine; TFU, Trifluridine; VDB, Vidarabine.

efficacy against FHV-1, followed by pharmacokinetic and safety trials in normal cats, subsequent placebo-controlled efficacy studies in experimentally inoculated animals, and, finally, carefully designed and monitored clinical trials in client-owned animals, is critical. The remainder of this review summarizes data from such studies.

Nucleoside or nucleotide analogues for topical administration
Idoxuridine (5-iodo-2'-deoxyuridine) is a thymidine analogue developed for treatment of humans infected with HSV-1.⁶ It differs from thymidine by having a single iodide substitution at position 5 on the pyrimidine ring. Following intracellular phosphorylation, it competes with thymidine for incorporation into viral DNA thus rendering the resultant virus incapable of replication. However, as a nonspecific inhibitor of DNA synthesis, idoxuridine affects any process requiring thymidine, and host cells are similarly affected. Therefore, systemic therapy is not possible, and corneal toxicity can occur.⁷ Where it is not commercially available, it can be obtained from a compounding pharmacy in formulations approximating those once FDA-approved for use in humans - i.e., as an ophthalmic solution (0.1%) or ointment (0.5%). In a retrospective case series of cats with ocular disease attributed to FHV-1, 0.1% idoxuridine solution was used every 4–6 h with improvement or resolution of clinical signs in 3 cats and no improvement or worsening in 4 cats.²

Vidarabine (adenine arabinoside; 9- β -D-arabinofuranosyladenine) is an adenosine analogue originally developed as a cancer chemotherapeutic⁸ but subsequently found to be efficacious against varicella zoster virus⁹ and HSV-1.¹⁰ Following triphosphorylation, vidarabine disrupts DNA synthesis via effects on DNA polymerase. Like idoxuridine, vidarabine is nonselective in its effect and associated with notable host toxicity - especially if administered systemically.¹¹ Because it affects a viral replication step different from that targeted by idoxuridine, vidarabine may be effective in patients whose disease appears resistant to idoxuridine.¹² As a 3% ophthalmic ointment, vidarabine often

Box 1. Some important general concepts about antiviral agents that may be used to guide prescribing and expectations when using these drugs

- Because viruses are less capable than are bacteria of independent function and because FHV-1 is a DNA virus, drug targets for antiviral agents are less numerous and tend to be less specific than they are for antibacterial agents.
- FHV-1 is an obligate intracellular organism which replicates within the host nucleus. Therefore, antiviral agents tend to be more toxic than antibacterial agents and often are sufficiently toxic that they may only be used topically and, even then, induce notable corneoconjunctival cytotoxicity.
- All antiviral agents to date are virostatic; therefore, they cannot target latent virus and must be administered frequently (systemically and topically).
- No antiviral drug has proven antibacterial activity.
- No antibacterial drug has proven antiviral activity.
- Antiviral drugs safe in humans are not necessarily safe in cats.
- Antiviral drugs effective against human herpesviruses are not necessarily effective against FHV-1.
- Antiviral prodrugs metabolized to their active form by humans are not predictably metabolized by cats.

appears to be better tolerated than many of the antiviral solutions including idoxuridine.¹³ Where it is not available commercially, it can be obtained from a compounding pharmacist. In a retrospective case series of cats with ocular disease attributed to FHV-1, 3% vidarabine ointment was used every 4 to 6 h with improvement noted in 1 cat and no improvement or worsening noted in 2 cats.²

Trifluridine (trifluorothymidine; 5-trifluoromethyl-2'-deoxyuridine) is a fluorinated nucleoside analogue of thymidine. Its specific mechanism of action against HSV-1 is not completely understood and has not been reported in FHV-1. However, following intracellular phosphorylation, it reduces DNA synthesis via inhibition of thymidylate synthetase. It is too toxic to be administered systemically but topically administered trifluridine is very effective at treating HSV-1 keratitis.¹³ This is in part due to its superior corneal epithelial penetration in comparison with idoxuridine and vidarabine.¹⁴ It is commercially

available as a 1% ophthalmic solution; however, it frequently causes marked ocular irritation in cats. In a retrospective case series of cats with ocular disease attributed to FHV-1, 1% trifluridine solution was used every 4–8 h with improvement in 1 cat and no improvement or worsening in 2 cats.²

Cidofovir (HPMPC; (S)-1-[3-hydroxy-2-(phosphonmethoxy)propyl]cytosine) is a cytosine analogue requiring 2 host-mediated but no virally-mediated phosphorylation steps.¹⁵ Its safety arises from its relatively high affinity for viral DNA polymerase compared with human DNA polymerase.¹⁶ Injectable cidofovir is administered intravenously or intravitreally to humans infected with herpesviruses, principally cytomegalovirus.^{17,18} Cidofovir applied as a 0.5% or 1.0% ophthalmic solution in rabbit models of human herpetic keratoconjunctivitis was equally effective when administered only twice daily as trifluridine administered 4–9 times daily,^{19,20} presumably due to the long tissue half-lives of cidofovir's metabolites.²¹ In a prospective, masked placebo-controlled study, a 0.5% ophthalmic solution of cidofovir compounded in methylcellulose and applied twice daily to cats experimentally infected with FHV-1 reduced viral shedding and clinical disease.²² However, nasolacrimal stenosis has been reported in humans receiving cidofovir topically,^{23,24} and it is not commercially available as an ophthalmic agent. Therefore, although the *in vitro*²⁵ and short-term *in vivo* efficacy²² of cidofovir against FHV-1 is proven, cats should be monitored for nasolacrimal cicatrization. Cidofovir 0.5% retained efficacy when compounded in normal saline and refrigerated (4 °C) or frozen (–20 or –80 °C) in plastic or glass for up to 6 months.²⁶ However, safety data including change in pH, tonicity, and risk of contamination were not evaluated.

Purine analogues and their oral prodrugs

Acyclovir (9-(2-hydroxyethoxy-methyl)guanine) is the prototype of a group of antiviral drugs known as acyclic nucleoside analogues with all members requiring 3 phosphorylation steps for activation. The first step must be catalyzed by viral thymidine kinase,²⁷ while the second and third phosphorylation steps must be performed by host enzymes. This increases the safety of the acyclic nucleosides and permits their systemic administration in humans.²⁸ However, FHV-1's thymidine kinase phosphorylates acyclovir much less efficiently than does the HSV-1-encoded enzyme, likely explaining the relative lack of efficacy of acyclovir against FHV-1 (see Table 1).^{29,30} To the authors' knowledge, the affinity of feline enzymes for the acyclic nucleoside analogues has not been reported. In addition to relatively low antiviral potency against FHV-1,^{25,31} acyclovir has poor bioavailability and can cause bone marrow suppression when systemically administered to cats.³² Oral administration of 50 mg/kg acyclovir to cats was associated with peak plasma concentrations of only 33 µM (approximately one third the IC₅₀ for

FHV-1).³² Thus, systemic acyclovir administration is not recommended in cats. Application of acyclovir as 0.5% ophthalmic ointment five times daily in cats with ocular disease attributable to FHV-1 led to resolution of clinical signs after 10 days in a nonmasked, nonplacebo-controlled study.³³ However, cats treated only three times daily took approximately twice as long to resolve and did so only once therapy was increased to five times daily. This suggests that at least five times daily topical application of acyclovir may produce corneal surface concentrations exceeding the IC₅₀ for FHV-1 without causing clinically appreciable toxicity.

Valacyclovir (L-valine 2-[(2-amino-1,6-dihydro-6-oxo-9H-purin-9-yl)methoxy]ethyl ester, monochloride) is a pro-drug developed to improve the bioavailability of acyclovir. In humans and cats, valacyclovir is more efficiently absorbed from the gastrointestinal tract than acyclovir is and, following absorption, is converted to acyclovir by a hepatic hydrolase.³⁴ Plasma concentrations of acyclovir exceeding the IC₅₀ for FHV-1 can be achieved after oral administration of this drug to cats. However, in cats experimentally infected with FHV-1, valacyclovir induced potentially fatal hepatic and renal necrosis, along with bone marrow suppression, without reducing viral shedding or clinical disease severity. This likely resulted from the toxic plasma concentrations of acyclovir that were achieved.³⁵ Valacyclovir should never be administered to cats.

Ganciclovir and valganciclovir. Ganciclovir (DHPG; 9-[[2-hydroxy-1-(hydroxymethyl)ethoxy]methyl]-guanine) is an acyclic nucleoside analogue with potent antiviral activity against HSV-1 and HSV-2.³⁶ It is approximately 10-fold more effective against FHV-1 *in vitro* than is acyclovir.²⁵ It is available for oral or intravenous administration in humans, where it is associated with more severe neurologic toxicity, neutropenia, and bacterial infections than is acyclovir.^{37,38} Additionally, an intravitreal sustained-release ganciclovir implant has been developed for treatment of cytomegalovirus retinitis in humans,³⁹ and a 0.15% ophthalmic gel is commercially available for treatment of acute human herpetic keratitis.⁴⁰ Valganciclovir (L-valine 2-[(2-amino-1,6-dihydro-6-oxo-9H-purin-9-yl)methoxy]-3-hydroxypropyl ester) is a prodrug of ganciclovir developed to address low oral bioavailability of ganciclovir,⁴¹ and prescribed to treat cytomegalovirus retinitis in humans.⁴² Although the *in vitro* efficacy of ganciclovir against FHV-1²⁵ and anecdotal reports of its topical administration to cats in Europe is very promising, to the authors' knowledge, the safety, efficacy, and pharmacokinetics of any formulation of valganciclovir or ganciclovir have not been reported in cats.

Penciclovir (9-(4-hydroxy-3-hydroxymethylbut-1-yl)guanine; BRL39123) is a nucleoside deoxyguanosine analogue with a similar mechanism of action to acyclovir and with potent antiviral activity against a number of human herpesviruses. Like acyclovir, it requires viral and cellular phosphorylation but is highly effective against FHV-1

in vitro^{29,43,44} and *in vivo*.⁴⁵ In a rabbit model of human HSV-1 keratitis, a 3% penciclovir ointment administered once, twice or four times daily decreased epithelial keratitis severity. Thus, a topical ophthalmic penciclovir ointment may be effective in cats with FHV-1 keratitis and/or conjunctivitis, but, to the authors' knowledge, there are no commercial or compounded preparations available for ophthalmic use.¹⁹ Penciclovir is available as a 1% dermatologic cream for humans, but that should not be applied to the eye.

Famciclovir (2-(2-(2-amino-9H-Purin-9-yl)ethyl)-1,3-propanediol diacetate) is a highly bioavailable prodrug of penciclovir which—once absorbed—is metabolized to penciclovir. In humans, this metabolism is complex, requiring di-deacetylation to BRL42359, in the blood, liver or small intestine, with subsequent oxidation to penciclovir by aldehyde oxidase in the liver (Fig. 1).^{46–48} Neither famciclovir nor BRL42359 has any *in vitro* antiviral activity against FHV-1;⁴³ therefore, complete metabolism to penciclovir is required. However, hepatic aldehyde oxidase activity in cats is about 2% of that seen in humans and lower than in any other species reported to date (Fig. 2).⁴⁹ Famciclovir pharmacokinetics in the cat are extremely complex and nonlinear (i.e., doubling of famciclovir dose does not lead to doubling of plasma penciclovir concentration) presumably due to saturation of the hepatic oxidase.⁵⁰ As a result, very high plasma concentrations of BRL42359 accumulate in the cat.⁵⁰ Fortunately, this compound demonstrates very little cytotoxicity *in vitro*.⁴³ Table 2 summarizes the pharmacokinetic data available to date for penciclovir in tears and plasma of cats receiving one of numerous famciclovir dose regimens. Tissue concentration data are not yet available.

In addition to these pharmacokinetic data, recommendation of an appropriate famciclovir dose requires the following:

- (1) Knowledge of whether penciclovir concentrations in plasma, tears, or the infected tissues themselves are most relevant.
- (2) Selection of an appropriate target penciclovir concentration based on reported *in vitro* IC₅₀ values which have ranged from 304 to 3500 ng/ml.^{25,31,43,44}
- (3) Knowledge of whether the targeted IC₅₀ should be exceeded by the trough or the peak penciclovir concentration and for how long.

Together, these uncertainties have led to much controversy about the optimum famciclovir dose in cats, with reported doses ranging from 8 mg/kg once daily⁵¹ to 140 mg/kg thrice daily.³ The following data are provided to inform dose selection.

In the only masked, placebo-controlled efficacy trial to date, cats experimentally inoculated with FHV-1 and given approximately 90 mg famciclovir/kg thrice daily *per os* achieved an approximate peak plasma penciclovir concentration of 2100 ng/ml.⁴⁵ Relative to control cats, treated cats had significantly reduced clinical signs, decreased serum globulin concentrations, reduced histologic evidence of conjunctivitis, decreased viral shedding and reduced serum FHV-1 titers, as well as increased goblet

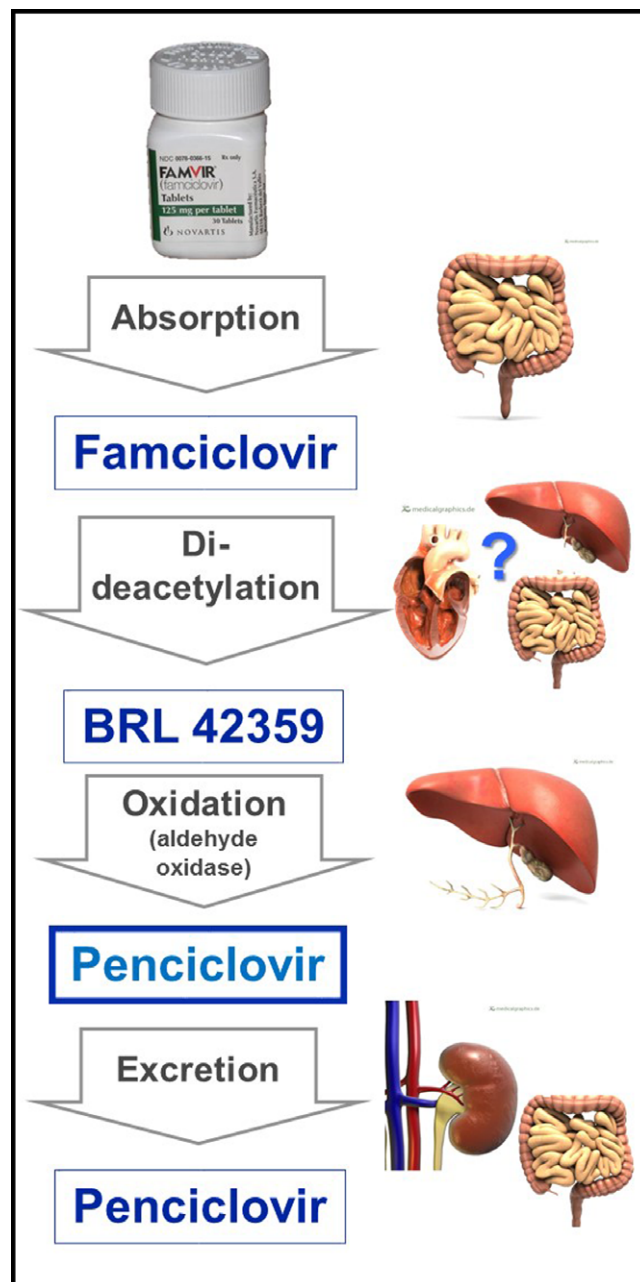


Figure 1. The absorption, metabolism, and excretion pathway of famciclovir in humans. Following oral administration, famciclovir is absorbed across the intestine and undergoes di-deacetylation. The exact site at which this step occurs is unclear but may be in the enterocytes, bloodstream, or liver. The inactive metabolite BRL42359 is then oxidized by a hepatic aldehyde oxidase to the active antiviral compound, penciclovir, which is ultimately excreted in feces and urine. Based upon famciclovir, BRL42359 and penciclovir concentrations in feline plasma following oral administration of famciclovir, similar steps likely occur in cats.^{50,52,53} Anatomic images courtesy of www.MedicalGraphics.de (license # CC BY-ND 3.0 DE).

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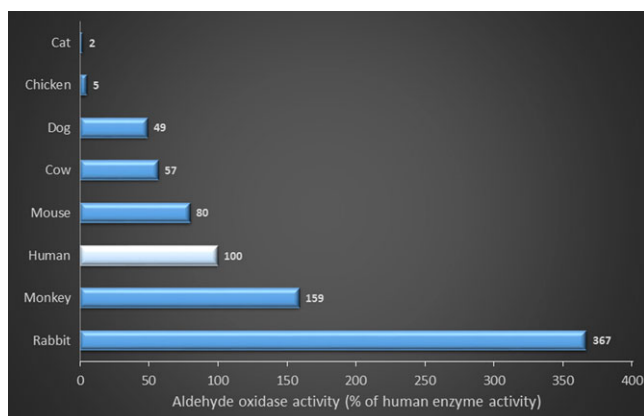


Figure 2. Relative activity of hepatic aldehyde oxidase in various species. All data are shown as percentage activity normalized against humans (100%). Original data from Dick *et al.*⁴⁹

cell density.⁴⁵ A subsequent study showed that administration of a single dose of 40 mg/kg to uninfected healthy cats achieved nearly identical plasma penciclovir concentrations to those achieved with a single dose of 90 mg/kg.⁵² A third study⁵³ revealed that client-owned cats with spontaneous disease administered famciclovir at 40 mg/kg thrice daily had tear penciclovir concentrations likely to be effective against FHV-1 for at least 3 h after each dose (i.e., for ≥ 9 h/day). This study used a target IC_{50} of 304 ng/ml.²⁹ In the most comprehensive pharmacokinetic study to date, healthy cats were administered famciclovir at 30, 40 or 90 mg/kg twice or thrice daily, and plasma and tear famciclovir, BRL42359, and penciclovir concentrations were measured.⁵⁰ This resulted in the recommendation that cats should receive 90 mg famciclovir/kg twice daily because this regimen achieved comparable plasma and tear penciclovir concentrations to those achieved with 90 mg/kg thrice daily, whereas the lower doses tested did not result in adequate tear penciclovir concentrations, even when administered thrice daily.

Perhaps the most clinically helpful data so far are from a retrospective study comparing outcomes when famciclovir was administered to 59 client-owned cats with presumed herpetic disease. All cats received famciclovir thrice daily at approximately 40 ($n = 33$ cats) or 90 mg/kg ($n = 26$ cats).³ Median duration of therapy required for clinical improvement was significantly longer in cats administered 40 vs. 90 mg/kg. Furthermore, cats in the 90 mg/kg group showed significantly greater and faster improvement than did cats in the 40 mg/kg group (Fig. 3). The reduction in treatment duration with the higher famciclovir dose was estimated to decrease overall client costs due to a reduction in total famciclovir administered, and potentially the number of recheck examinations required. Therefore, although clinical data suggest that 90 mg/kg TID is highly efficacious and cost-effective, pharmacokinetic data⁵⁰ suggest that tear and plasma penciclovir concentrations are similar whether cats receive 90 mg famciclovir/kg 2 or 3 times daily. Taken together, data from these two studies^{3,50} therefore suggest that 90 mg famciclovir/kg twice daily is likely to be effective in treating cats with herpetic disease. In the non-controlled clinical trial, adverse events potentially attributable to famciclovir (most commonly gastrointestinal) were reported in 17% of cats receiving 40 or 90 mg famciclovir/kg thrice daily, but the prevalence was not different between the two dose groups.³ Assessing all *in vivo* tolerance data for famciclovir, this drug appears to be markedly safer than acyclovir and valacyclovir—the only other systemic antiviral drugs for which there are reports of oral administration to cats.^{3,32,35,45,50,52–54} However, patients administered famciclovir should be closely monitored, and assessment of a complete blood count, serum biochemistry panel and urinalysis should be considered in cats with known concurrent disease or cats expected to receive famciclovir for long periods. As in humans,⁵⁵ reduction in dose frequency should be considered in cats with renal insufficiency.^{3,50}

Table 2. Maximum (C_{max}) and minimum plasma and tear penciclovir concentrations and time to plasma and tear C_{max} in cats administered a variety of famciclovir doses at various dose frequencies

Famciclovir dose*	Dose frequency	Plasma penciclovir concentration (ng/ml)		Plasma penciclovir T_{max} (h)	Tear penciclovir concentration (ng/ml)		Tear penciclovir T_{max} (h)
		C_{max}	$C_{ss(min)}$		C_{max}	$C_{ss(min)}$	
9–18 mg/kg ⁵⁴	BID	330	64	5.3	ND	ND	ND
	TID	680	180	3.8	ND	ND	ND
30 mg/kg ⁵⁰	BID	2010	345	1.3	305	65	4.7
	TID	1755	570	1.7	395	160	2.0
40 mg/kg ⁵⁰	BID	1945	445	2.3	375	55	3.3
	TID	2210	790	2.5	750	150	2.6
90 mg/kg ⁵⁰	BID	2720	630	2.7	680	200	2.3
	TID	2015	905	2.7	555	275	3.0

* Superscript numbers are cited references.

BID, twice daily; C_{max} , maximum observed drug concentration; $C_{ss(min)}$, minimum observed drug concentration during the dosing interval at steady state; ND, not done; TID, thrice daily; T_{max} , Time to maximum observed drug concentration.

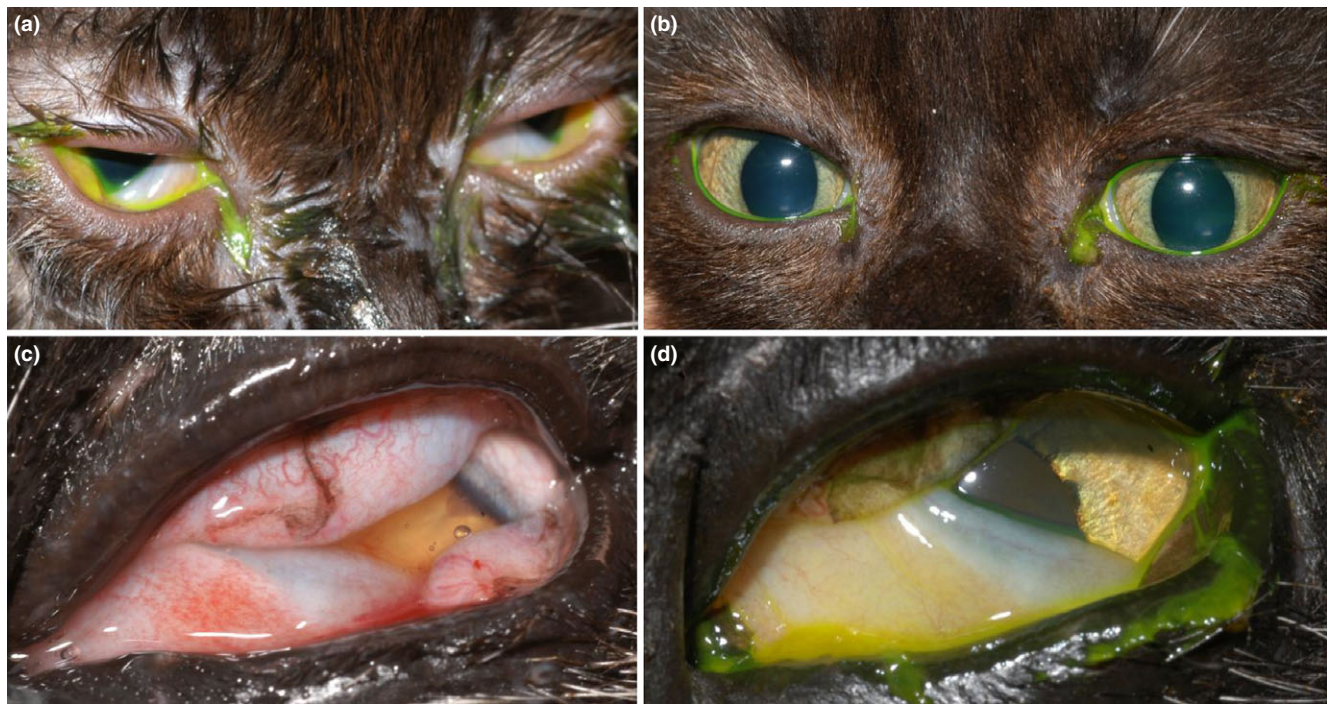


Figure 3. A 6-week-old, sexually intact male domestic short-haired cat with blepharoconjunctivitis in both eyes and dendritic ulcerative keratitis in the right eye prior to (a) and following (b) 8 days of orally administered famciclovir given at 110 mg/kg thrice daily. Note the marked improvement in both eyes following treatment with famciclovir. Left eye of a 14-year-old, female spayed domestic medium-haired cat with blepharokeratoconjunctivitis in both eyes prior to (c) and following (d) 15 days of orally administered famciclovir given at 85 mg/kg thrice daily. Note the improvement in the left eye following treatment with famciclovir.

Other antiviral drugs

Foscarnet (phosphonoformate) mimics the anion pyrophosphate to selectively inhibit the pyrophosphate-binding site on viral DNA polymerases at concentrations that do not affect human DNA polymerases.⁵⁶ Foscarnet is administered intravenously to treat cytomegalovirus retinitis or mucocutaneous acyclovir-resistant HSV infections in immunocompromised humans.⁵⁷ However, foscarnet has very low oral bioavailability (8%) in cats⁵⁷ and markedly lower *in vitro* activity against FHV-1 in comparison with most other antiviral drugs reported.²⁵ Therefore, its use in cats is not recommended.

Bromovinyldeoxyuridine,^{31,58} *adefovir*,⁵⁹ *PMEDAP* (9-(2-phosphonylmethoxyethyl)-2, 6-diaminopurine),⁵⁹ and *HPMPA* ((S)-9-(3-hydroxy-2-phosphonylmethoxypropyl) adenine)³¹ have variable *in vitro* efficacy against FHV-1 (see Table 1). To the authors' knowledge, their efficacy and safety when administered orally or topically to cats have not been reported.

OTHER COMPOUNDS INVESTIGATED FOR ACTIVITY AGAINST FHV-1

Lysine is perhaps the best studied and yet maybe one of the more controversial of all of the other compounds with proven or putative antiviral efficacy against FHV-1 in cats.

As with the antiviral drugs, initial interest arose from *in vitro* data and clinical trials in humans. Lysine's antiviral effect is believed to arise because arginine is an essential amino acid for FHV-1⁶⁰ and HSV-1^{61,62} replication, and assumes that lysine antagonizes arginine availability to or utilization by these viruses during protein synthesis. This was originally hypothesized to affect protein synthesis of the virus more than the host because viral proteins had a higher arginine-to-lysine content than did human (and feline) proteins⁶³; however, recent analysis suggests that the difference in feline vs. FHV-1 protein amino acid content is minimal.⁶⁴ Markedly elevated lysine concentrations in combination with notably low arginine concentrations suppress HSV-1^{61,62} and FHV-1⁶⁰ replication *in vitro*. However, this was not borne out with more physiologic amino acid concentrations.⁶⁵ *In vivo* data in cats are also contradictory. Oral administration of 500 mg L-lysine every 12 h beginning 6 h prior to inoculation with FHV-1 was associated with less severe conjunctivitis but similar viral shedding to that seen in cats receiving placebo.⁶⁶ In cats latently infected following experimental inoculation but without clinical signs at the time of the study, oral administration of 400 mg L-lysine once-daily reduced viral shedding relative to placebo-treated cats.⁶⁷ Despite significant elevations in the cats' plasma lysine concentration, no change in plasma arginine concentration

was observed in either study. Mild, reversible gastrointestinal disturbance potentially attributable to lysine administration was noted in some cats.⁶⁶ In the only study to assess bolus administration of lysine in naturally infected cats, 144 shelter-housed cats received 250 mg (kittens) or 500 mg (adult cats) lysine once daily for their entire shelter stay; outcomes were compared with an untreated control group. No significant treatment effect was detected for any parameter.⁶⁸

Safety and efficacy of dietary lysine supplementation have also been assessed. No ill effects were seen in cats fed diets supplemented to up to 8.6% (dry matter) lysine.⁶⁹ In 2 subsequent efficacy trials, cats in environments where FHV-1 was enzootic were fed a diet supplemented to 5.1% lysine, while control cats received a basal ration (approximately 1% lysine).^{70,71} Paradoxically and in both studies, disease was more severe and viral shedding was increased in cats fed the supplemented ration relative to those fed the basal diet. This may be partially explained by the observation that cats decreased their food (and therefore lysine) intake coincident with peak disease and viral presence.⁷⁰

In summary, there is considerable variability among these studies, especially with respect to methodology, study population, and dose and method of lysine administration. However, taken together, data from these studies suggest that lysine is safe when orally administered to cats and, provided that it is administered as a bolus, may reduce viral shedding in latently infected cats and clinical signs in cats undergoing primary exposure to the virus. However, the stress of bolus administration in shelter situations may well negate its effects and data do not support dietary supplementation in any cats. Unfortunately, no clinical trials have been conducted on the group in which this drug is commonly used—client-owned cats with recurrent herpetic disease.

Interferons (IFNs) are cytokines with diverse immunological and antiviral functions; they may be divided into four types (α , β , γ , and ω) and numerous subtypes. Viral infection stimulates cells to secrete IFNs into the extracellular space where they limit viral spread to adjacent cells without being virucidal. This knowledge should be used to set reasonable expectations of how therapeutically efficacious IFNs may be and to decide in which patients and at what stages of disease they might be expected to be most effective.

Although IFNs likely play important physiologic roles in the control of viral infections, *in vitro* data and clinical trials have produced conflicting and generally unsupportive results. *In vitro* tests using 1×10^5 to 5×10^5 IU/ml of recombinant human IFN- α or feline IFN- ω reduced FHV-1 titer and/or cytopathic effect without observable cytotoxicity to the feline corneal cell line⁷² or Crandell-Rees feline kidney (CRFK) cells⁷³ on which the virus was grown. At higher concentrations, the effect of IFN- ω was greater than that of IFN- α .⁷³ In another *in vitro* study,

notable synergistic activity against FHV-1 was demonstrated when 10–62.5 μ g/ml of acyclovir was combined with 10 or 100 IU/ml of human recombinant IFN- α . The combination did not increase cytotoxicity but permitted a nearly eightfold reduction in acyclovir dose required to achieve maximal FHV-1 inhibition. Although synergy occurred when the IFN- α was given before or after infection, pretreatment was more effective.⁷⁴ These data are supported by a study using a murine model of HSV-1 whereby concurrent oral acyclovir and intraperitoneal recurrent human IFN- α was more efficacious than either treatment alone.⁷⁵ *In vivo* investigation of nucleoside analogues in combination with IFN in cats is warranted, however, before their use can be recommended.

To the authors' knowledge, there have been only two experimental FHV-1 inoculation studies in which IFNs have been studied. In the first, 5 SPF cats were pretreated with 10 000 IU of recombinant feline IFN- ω OU q 12 h and 2000 IU administered PO q 24 h for 2 days prior to viral inoculation; IFN therapy was not continued after inoculation.⁷⁶ No beneficial effects were shown. In the second study, twice daily subcutaneous administration of 10^8 IU/kg IFN- α on two consecutive days prior to inoculation did lead to lower cumulative clinical scores for treated cats.⁷⁷ In clinical trials, there are reports of IFN administration to 37 client-owned⁷⁸ and 13 shelter-housed⁷⁹ cats testing negative for FeLV and FIV, 24 shelter-housed cats testing negative for FeLV \pm FIV,⁸⁰ and 16 shelter-housed cats testing positive for FeLV, FIV, or both.⁸¹ These cats were of widely ranging ages and showed signs of acute,⁷⁸ unrecorded,^{80,81} or chronic unresponsive,⁷⁹ spontaneously occurring upper respiratory disease. They were treated with recombinant human IFN- α at 10 000 U/kg subcutaneously once daily for 14 days,⁷⁹ three 5-day cycles of once-daily subcutaneous injections of 1 million U/kg recombinant feline IFN- ω on days 0, 14, and 60,⁸¹ 1 drop of 1 million U/ml recombinant feline IFN- ω or human IFN- α OU twice daily for 14 days,⁸⁰ or 2.5 million units of recombinant IFN- ω injected subcutaneously once on Day 0 followed by 0.5 million units applied every 8 h for 21 days in each nostril and conjunctival sac (1 drop each) and the oral cavity (the remainder).⁷⁸ Only 2 of the studies were placebo-controlled; neither showed a significant treatment effect.^{78,80} Taken together, the data to date are not strongly supportive of interferon use in the management of herpetic disease in cats.

Lambda-carrageenan (λ -carrageenan) is a seaweed extract containing sulfated polysaccharides with *in vitro* activity against FHV-1 replication when used prior to but not following viral adsorption.⁸² *In vivo* safety and efficacy of λ -carrageenan were examined in a placebo-controlled, masked study in vaccinated cats exposed for the first time to wild-type FHV-1.⁸² Although well-tolerated, ophthalmic application of 1 drop of a 250 μ g/ml λ -carrageenan solution before and after infection ($n = 6$ cats) or

after infection only ($n = 6$ cats) did not reduce clinical signs. Reduction in viral shedding was noted only on Day 21 following inoculation. Other plant extracts with antiviral activity have undergone preliminary *in vitro* assessment, but clinical safety or efficacy has not been reported.⁸³

Leflunomide is an immunosuppressive agent with some antiviral efficacy against human herpesviruses.⁸⁴ *In vitro* efficacy studies with FHV-1 revealed significant and dose-dependent reduction in plaque number and—at higher concentrations only—viral load. However, at higher concentrations, some cytotoxicity was observed. Electron microscopy suggested a failure in viral tegument and external membrane assembly, which may indicate the mode of action.⁸⁵ Clinical studies are lacking.

Lactoferrin is a mammalian iron-binding glycoprotein that has antibacterial, antifungal, antiprotozoal, and antiviral properties. It is produced by mucosal epithelial cells and is present in tears and other body fluids. Lactoferrin has potent antiviral efficacy against FHV-1 replication *in vitro*, apparently via inhibition of adsorption or penetration of the virus into the cell.⁸⁶ Studies assessing the clinical relevance of these data are required.

Small interfering RNAs (siRNAs) are short (about 20-nucleotide), double-stranded sections of RNA designed to transfect a cell and reduce expression of specific genes. To overcome the short-lived effect of transfection with native siRNAs, they can be incorporated into plasmids and thus extend their longevity, especially within rapidly dividing cells. Initial *in vitro* studies demonstrated antiviral activity of siRNAs targeting the FHV-1 glycoprotein D (gD) alone or the gD and DNA polymerase genes jointly, but not the DNA polymerase gene alone.^{87,88} However, intracellular delivery of these agents is essential but proving complex. Agents that facilitate siRNA delivery into corneal cells *in vitro* have been developed and they appear nontoxic *in vitro* and nonirritating when applied topically to normal cats' eyes.⁸⁹ However, thus far, they have failed to deliver the siRNAs into corneal cells following topical application *in vivo*, perhaps due to rapid removal of the test substances from the ocular surface by tears.⁸⁹

Probiotics were investigated in a prospective, placebo-controlled, pilot study⁹⁰ in which cats experimentally infected with FHV-1 for another study²² were administered the probiotic *Enterococcus faecium* strain SF68. This clinical trial failed to reveal a significant treatment effect; however, cats in both groups showed such minimal evidence of disease that a treatment effect may have been missed.

SUMMARY

This review summarizes the current state of knowledge regarding antiviral drugs and other potentially antiviral compounds in cats. It is not a 'how to' manual for the

treatment of the diverse range of clinical herpetic syndromes. However, some general comments are possible:

- (1) All antiviral drugs studied to date are virostatic and so cannot be used to cure infection, only to reduce replicating virus, and thereby the severity, duration, or both of clinical signs associated with infection.
- (2) FHV-1 causes long-term and marked reduction in conjunctival goblet cell density⁹¹ that famciclovir only partially mitigates.⁴⁵ As a result, topical application of a mucinomimetic agent such as hyaluronate is often required as an adjunct to antiviral therapy.⁹²
- (3) Because antiviral drugs are virostatic, frequency of application of a topical agent and dose and frequency of a systemic agent are critical to therapeutic success.
- (4) *In vitro* selection of drug-resistant herpesviruses is performed by exposure to antiviral drug concentrations known to be ineffective.⁹³ Likewise, current guidelines for responsible antimicrobial stewardship reinforce the importance of appropriate dosing.⁹⁴ Under-dosing of antiviral agents, therefore, is likely to induce resistant viruses.
- (5) There are no clear guidelines regarding duration of therapy or, more specifically, when antiviral agents should be initiated and stopped. However, it appears reasonable that antiviral agents should be considered when signs are severe, persistent or recurrent, particularly when there is corneal involvement, and especially ulceration. Because epithelial replication, latency, and reactivation, and persistence are such interdependent and sequential phases of herpetic disease, interruption of any one of them is expected to limit the virus' abilities to cause subsequent disease. Therefore, aggressive treatment of herpetic disease may limit disease progression and minimize frequency and severity of recurrences. Likewise, prudent antimicrobial stewardship would suggest that therapy should be continued for a period after clinical signs are absent. The length of this period should be tailored to the individual based, in part, upon response to therapy, and duration and severity of the signs prior to treatment. Tapering of topically applied antiviral agents must be done with appropriate consideration for their lowest effective frequency as virostatic agents. For example, the recommended reduction in trifluridine dose as human herpetic keratitis improves is from 9 to 5 times daily, but not lower.⁹⁵ By comparison, tapering of orally administered antiviral drugs is never advised in acute herpetic syndromes, but is practiced in some herpes prophylaxis regimens. And then, the drug is reduced only to a dose proven to be effective.⁹⁵ Even in human patients with renal impairment and in whom metabolism of systemically administered antiviral agents is expected to be reduced, dose magnitude reduction is not recommended; rather, reduced dose frequency is preferred.⁹⁵ Indeed, there is good evidence that reduc-

tion or tapering of antiviral dose leads to a resurgence in the herpesviral fraction of the microbiome.⁹⁶ There are no data to support dose reduction or tapering of antiviral drugs in cats.

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