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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-arabinofuranasyladenine) is an adenosine analogue originally developed as a cancer chemotherapeutic[8] but subsequently found to be efficacious against varicella zoster virus[9] 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 thymidilate 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-(phosphono-methoxy)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* efficacy[22] 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-methy)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 IC$_{50}$ 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 IC$_{50}$ 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 prodrug 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 IC$_{50}$ 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-1[25] 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* IC$_{50}$ values which have ranged from 304 to 3500 ng/ml.[25,31,43,44]
(3) Knowledge of whether the targeted IC$_{50}$ 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 daily[51] 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, trea-

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).

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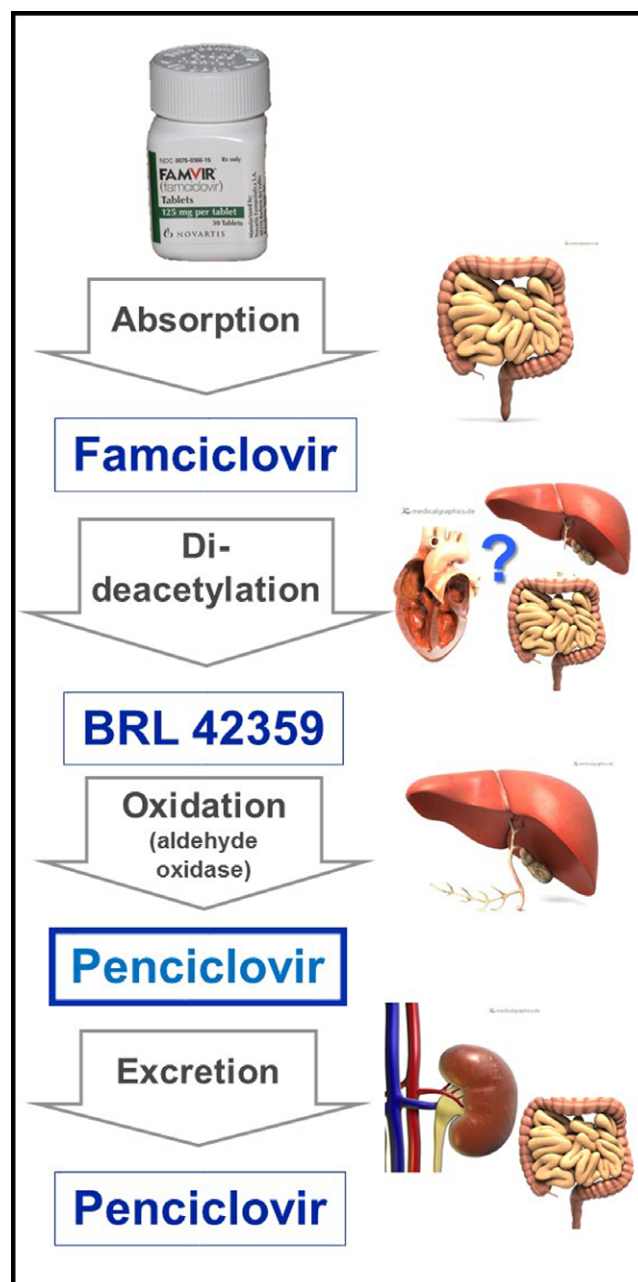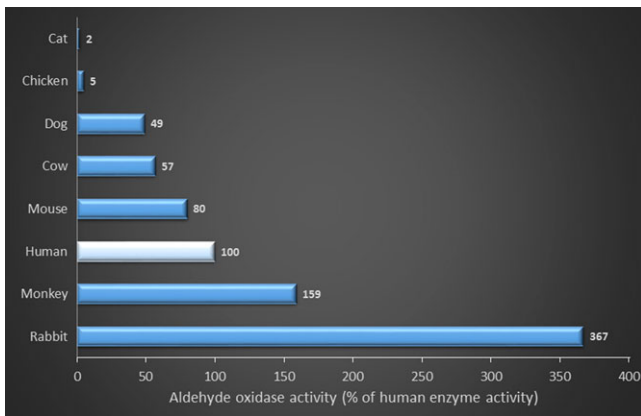
Figure 2. Relative activity of hepatic aldehyde oxidase in various species. All data are shown as percentage activity normalized against humans (100%). Original data from Dick et al.[49]

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 study[53] 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 data[50] 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.
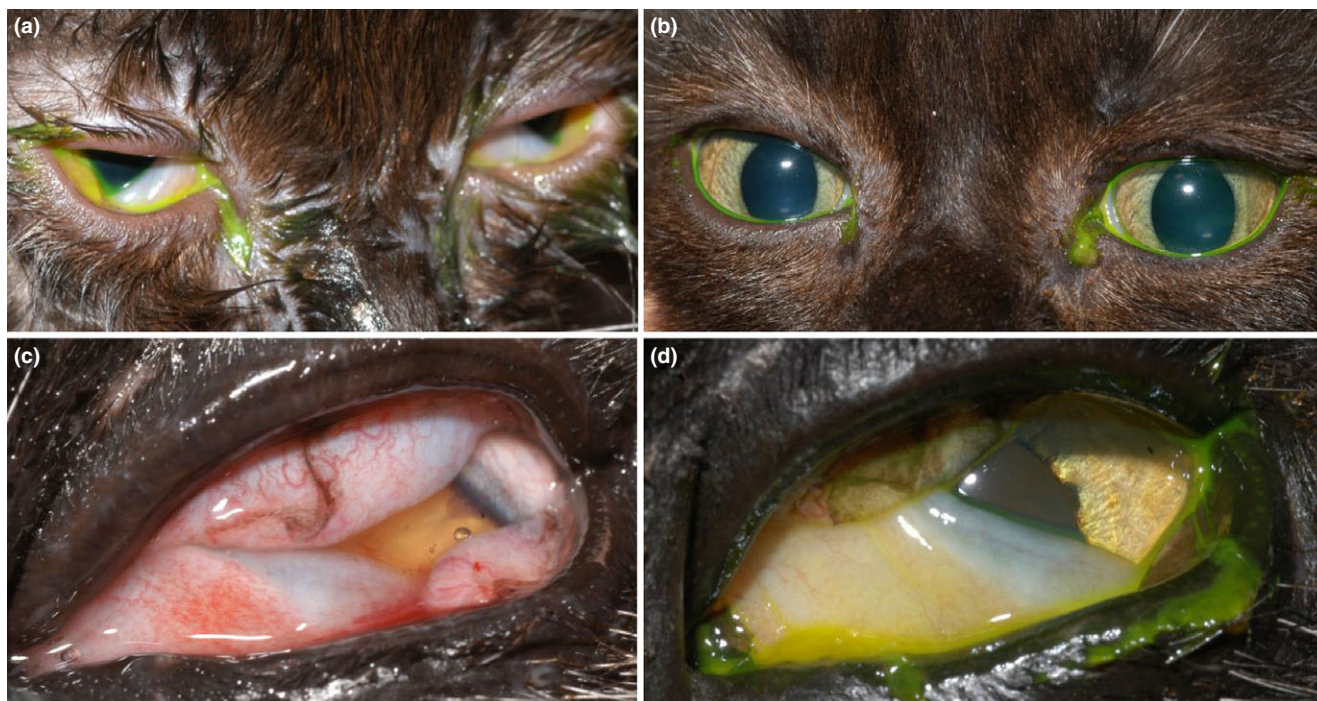
Figure 3. A 6-week-old, sexually intact male domestic short-haired cat with blepharoconjunctivitis in both eyes and dendritic ulcerative keratitis in the right eye prior to (a) and following (b) 8 days of orally administered famciclovir given at 110 mg/kg thrice daily. Note the marked improvement in 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 cats[57] 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-1[60] 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) proteins[63]; 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-1[60] 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 line[72] or Crandell-Rees feline kidney (CRFK) cells[73] 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 μg/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-owned[78] and 13 shelter-housed[79] cats testing negative for FeLV and FIV, 24 shelter-housed cats testing negative for FeLV ± 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 μg/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 study[90] in which cats experimentally infected with FHV-1 for another study[22] 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 density[91] 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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REFERENCES

1. Maggs DJ. Update on pathogenesis, diagnosis, and treatment of feline herpesvirus type 1. *Clinical Techniques in Small Animal Practice* 2005; **20**: 94–101.
2. Stiles J. Treatment of cats with ocular disease attributable to herpesvirus infection: 17 cases (1983–1993). *Journal of the American Veterinary Medical Association* 1995; **207**: 599–603.
3. Thomasy SM, Shull O, Outerbridge CAL *et al*. Oral administration of famciclovir for treatment of spontaneous ocular, respiratory or dermatologic disease attributed to feline herpesvirus type-1: a retrospective review in 59 client-owned cats. *Journal of the American Veterinary Medical Association* 2016; in press.
4. De Clercq E. Antivirals and antiviral strategies. *Nature Reviews Microbiology* 2004; **2**: 704–720.
5. James SH, Prichard MN. Current and future therapies for herpes simplex virus infections: mechanism of action and drug resistance. *Current Opinion in Virology* 2014; **8**: 54–61.
6. Luntz MH, MacCallum FO. Treatment of herpes simplex keratitis with 5-iodo-2'-deoxyuridine. *British Journal of Ophthalmology* 1963; **47**: 449–456.
7. Kaufman HE. Antimetabolite drug therapy in herpes simplex. *Ophthalmology* 1980; **87**: 135–139.
8. LePage GA, Khaliq A, Gottlieb JA. Studies of 9-beta-D-arabinofuranosyladenine in man. *Drug Metabolism and Disposition* 1973; **1**: 756–759.
9. Johnson MT, Buchanan RA, Luby JP *et al*. Treatment of varicella-zoster virus infections with adenine arabinoside. *Journal of Infectious Diseases* 1975; **131**: 225–229.
10. Brightbill FS, Kaufman HE. Adenine arabinoside therapy in corneal stromal disease and iritis due to herpes simplex. *Annals of Ophthalmology* 1974; **6**: 25–32.
11. Lauter CB, Bailey EJ, Lerner AM. Microbiologic assays and neurological toxicity during use of adenine arabinoside in humans. *Journal of Infectious Diseases* 1976; **134**: 75–79.
12. Nesburn AB, Robinson C, Dickinson R. Adenine arabinoside effect on experimental idoxuridine-resistant herpes simplex infection. *Investigative Ophthalmology* 1974; **13**: 302–304.
13. Chin GN. Treatment of herpes simplex keratitis with idoxuridine and vidarabine: a double-blind study. *Annals of Ophthalmology* 1978; **10**: 1171–1174.
14. O'Brien WJ, Edelhauser HF. The corneal penetration of trifluorothymidine, adenine arabinoside, and idoxuridine: a comparative study. *Investigative Ophthalmology & Visual Science* 1977; **16**: 1093–1103.
15. Cihlar T, Chen MS. Identification of enzymes catalyzing two-step phosphorylation of cidofovir and the effect of cytomegalovirus infection on their activities in host cells. *Molecular Pharmacology* 1996; **50**: 1502–1510.
16. Neyts J, Snoeck R, Schols D *et al*. Selective inhibition of human cytomegalovirus DNA synthesis by (S)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine [(S)-HPMPC] and 9-(1,3-dihydroxy-2-propoxymethyl)guanine (DHPG). *Virology* 1990; **179**: 41–50.
17. Bronson JJ, Ferrara LM, Hitchcock MJ *et al*. (S)-1-(3-hydroxy-2-(phosphonylmethoxy)propyl)cytosine (HPMPC): a potent antiherpesvirus agent. *Advances in Experimental Medicine and Biology* 1990; **278**: 277–283.
18. Snoeck R, Sakuma T, De Clercq E *et al*. (S)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine, a potent and selective inhibitor of human cytomegalovirus replication. *Antimicrobial Agents and Chemotherapy* 1988; **32**: 1839–1844.
19. Kaufman HE, Varnell ED, Thompson HW. Trifluridine, cidofovir, and penciclovir in the treatment of experimental herpetic keratitis. *Archives of Ophthalmology* 1998; **116**: 777–780.
20. Romanowski EG, Bartels SP, Gordon YJ. Comparative antiviral efficacies of cidofovir, trifluridine, and acyclovir in the HSV-1 rabbit keratitis model. *Investigative Ophthalmology & Visual Science* 1999; **40**: 378–384.
21. Neyts J, Snoeck R, Balzarini J *et al*. Particular characteristics of the anti-human cytomegalovirus activity of (S)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine (HPMPC) in vitro. *Antiviral Research* 1991; **16**: 41–52.
22. Fontenelle JP, Powell CC, Veir JK *et al*. Effect of topical ophthalmic application of cidofovir on experimentally induced primary ocular feline herpesvirus-1 infection in cats. *American Journal of Veterinary Research* 2008; **69**: 289–293.
23. Sherman MD, Feldman KA, Farahmand SM *et al*. Treatment of conjunctival squamous cell carcinoma with topical cidofovir. *American Journal of Ophthalmology* 2002; **134**: 432–433.
24. Gordon YJ, Romanowski EG, Araullo-Cruz T. Topical HPMPC inhibits adenovirus type 5 in the New Zealand rabbit ocular replication model. *Investigative Ophthalmology & Visual Science* 1994; **35**: 4135–4143.
25. Maggs DJ, Clarke HE. In vitro efficacy of ganciclovir, cidofovir, penciclovir, foscarnet, idoxuridine, and acyclovir against feline herpesvirus type-1. *American Journal of Veterinary Research* 2004; **65**: 399–403.
26. Stiles J, Gwin W, Pogranichniy R. Stability of 0.5% cidofovir stored under various conditions for up to 6 months. *Veterinary Ophthalmology* 2010; **13**: 275–277.
27. Darby G, Larder BA, Bastow KF *et al*. Sensitivity of viruses to phosphorylated 9-(2-hydroxyethoxymethyl)guanine revealed in TK-transformed cells. *Journal of General Virology* 1980; **48**: 451–454.
28. Whitley RJ. The past as prelude to the future: history, status, and future of antiviral drugs. *Annals of Pharmacotherapy* 1996; **30**: 967–971.
29. Hussein IT, Menashy RV, Field HJ. Penciclovir is a potent inhibitor of feline herpesvirus-1 with susceptibility determined at the level of virus-encoded thymidine kinase. *Antiviral Research* 2008; **78**: 268–274.
30. Hussein IT, Miguel RN, Tiley LS *et al*. Substrate specificity and molecular modelling of the feline herpesvirus-1 thymidine kinase. *Archives of Virology* 2008; **153**: 495–505.
31. Williams DL, Fitzmaurice T, Lay L *et al*. Efficacy of antiviral agents in feline herpetic keratitis: results of an in vitro study. *Current Eye Research* 2004; **29**: 215–218.
32. Owens JG, Nasisse MP, Tadepalli SM *et al*. Pharmacokinetics of acyclovir in the cat. *Journal of Veterinary Pharmacology and Therapeutics* 1996; **19**: 488–490.
33. Williams DL, Robinson JC, Lay E *et al*. Efficacy of topical aciclovir for the treatment of feline herpetic keratitis: results of a prospective clinical trial and data from in vitro investigations. *The Veterinary Record* 2005; **157**: 254–257.

34. de Miranda P, Burnette TC. Metabolic fate and pharmacokinetics of the acyclovir prodrug valaciclovir in cynomolgus monkeys. *Drug Metabolism and Disposition* 1994; **22**: 55–59.

35. Nasisse MP, Dorman DC, Jamison KC *et al.* Effects of valacyclovir in cats infected with feline herpesvirus 1. *American Journal of Veterinary Research* 1997; **58**: 1141–1144.

36. Smith KO, Galloway KS, Kennell WL *et al.* A new nucleoside analog, 9-[[2-hydroxy-1-(hydroxymethyl)ethoxy]methyl]guanine, highly active in vitro against herpes simplex virus types 1 and 2. *Antimicrobial Agents and Chemotherapy* 1982; **22**: 55–61.

37. Ernst ME, Franey RJ. Acyclovir- and ganciclovir-induced neurotoxicity. *Annals of Pharmacotherapy* 1998; **32**: 111–113.

38. Burns LJ, Miller W, Kandaswamy C *et al.* Randomized clinical trial of ganciclovir vs acyclovir for prevention of cytomegalovirus antigenemia after allogeneic transplantation. *Bone Marrow Transplantation* 2002; **30**: 945–951.

39. Anand R, Nightingale SD, Fish RH *et al.* Control of cytomegalovirus retinitis using sustained release of intraocular ganciclovir. *Archives of Ophthalmology* 1993; **111**: 223–227.

40. Chou TY, Hong BY. Ganciclovir ophthalmic gel 0.15% for the treatment of acute herpetic keratitis: background, effectiveness, tolerability, safety, and future applications. *Therapeutics and Clinical Risk Management* 2014; **10**: 665–681.

41. Jung D, Dorr A. Single-dose pharmacokinetics of valganciclovir in HIV- and CMV-seropositive subjects. *Journal of Clinical Pharmacology* 1999; **39**: 800–804.

42. Segarra-Newnham M, Salazar MI. Valganciclovir: a new oral alternative for cytomegalovirus retinitis in human immunodeficiency virus-seropositive individuals. *Pharmacotherapy* 2002; **22**: 1124–1128.

43. Groth AD, Contreras MT, Kado-Fong HK *et al.* In vitro cytotoxicity and antiviral efficacy against feline herpesvirus type 1 of famciclovir and its metabolites. *Veterinary Ophthalmology* 2014; **17**: 268–274.

44. Hussein IT, Field HJ. Development of a quantitative real-time TaqMan PCR assay for testing the susceptibility of feline herpesvirus-1 to antiviral compounds. *Journal of Virological Methods* 2008; **152**: 85–90.

45. Thomasy SM, Lim CC, Reilly CM *et al.* Evaluation of orally administered famciclovir in cats experimentally infected with feline herpesvirus type-1. *American Journal of Veterinary Research* 2011; **72**: 85–95.

46. Clarke SE, Harrell AW, Chenery RJ. Role of aldehyde oxidase in the in vitro conversion of famciclovir to penciclovir in human liver. *Drug Metabolism and Disposition* 1995; **23**: 251–254.

47. Vere Hodge RA, Sutton D, Boyd MR *et al.* Selection of an oral prodrug (BRL 42810; famciclovir) for the antiherpesvirus agent BRL 39123 [9-(4-hydroxy-3-hydroxymethylbut-l-yl)guanine; penciclovir]. *Antimicrobial Agents and Chemotherapy* 1989; **33**: 1765–1773.

48. Filer CW, Allen GD, Brown TA *et al.* Metabolic and pharmacokinetic studies following oral administration of 14C-famciclovir to healthy subjects. *Xenobiotica* 1994; **24**: 357–368.

49. Dick RA, Kanne DB, Casida JE. Identification of aldehyde oxidase as the neonicotinoid nitroreductase. *Chemical Research in Toxicology* 2005; **18**: 317–323.

50. Sebbag L, Thomasy SM, Woodward AP *et al.* Pharmacokinetic modeling of penciclovir and BRL42359 in plasma and tears so as to optimize oral famciclovir dosing recommendation in cats. *American Journal of Veterinary Research* 2016; in press.

51. Malik R, Lessels NS, Webb S *et al.* Treatment of feline herpesvirus-1 associated disease in cats with famciclovir and related drugs. *Journal of Feline Medicine and Surgery* 2009; **11**: 40–48.

52. Thomasy SM, Whittem T, Bales JL *et al.* Pharmacokinetics of penciclovir in healthy cats following oral administration of famciclovir or intravenous infusion of penciclovir. *American Journal of Veterinary Research* 2012; **73**: 1092–1099.

53. Thomasy SM, Covert JC, Stanley SD *et al.* Pharmacokinetics of famciclovir and penciclovir in tears following oral administration of famciclovir to cats: a pilot study. *Veterinary Ophthalmology* 2012; **15**: 299–306.

54. Thomasy SM, Maggs DJ, Moulin NK *et al.* Pharmacokinetics and safety of penciclovir following oral administration of famciclovir to cats. *American Journal of Veterinary Research* 2007; **68**: 1252–1258.

55. Boike SC, Pue MA, Freed MI *et al.* Pharmacokinetics of famciclovir in subjects with varying degrees of renal impairment. *Clinical Pharmacology and Therapeutics* 1994; **55**: 418–426.

56. Hess G, Arnold W. Meyer zum Buschenfelde KH. Inhibition of hepatitis-B-virus DNA polymerase by phosphonoformate: studies on its mode of action. *Journal of Medical Virology* 1980; **5**: 309–316.

57. De Clercq E. Selective anti-herpesvirus agents. *Antiviral Chemistry & Chemotherapy* 2013; **23**: 93–101.

58. Nasisse MP, Guy JS, Davidson MG *et al.* In vitro susceptibility of feline herpesvirus-1 to vidarabine, idoxuridine, trifluridine, acyclovir, or bromovinyldeoxyuridine. *American Journal of Veterinary Research* 1989; **50**: 158–160.

59. van der Meulen K, Garre B, Croubels S *et al.* In vitro comparison of antiviral drugs against feline herpesvirus 1. *BMC Veterinary Research* 2006; **2**: 13.

60. Maggs DJ, Collins BK, Thorne JG *et al.* Effects of L-lysine and L-arginine on in vitro replication of feline herpesvirus type-1. *American Journal of Veterinary Research* 2000; **61**: 1474–1478.

61. Tankersley RW Jr. Amino acid requirements of herpes simplex virus in human cells. *Journal of Bacteriology* 1964; **87**: 609–613.

62. Griffith RS, DeLong DC, Nelson JD. Relation of arginine-lysine antagonism to herpes simplex growth in tissue culture. *Chemotherapy* 1981; **27**: 209–213.

63. Kaplan AS, Shimono H, Ben-Porat T. Synthesis of proteins in cells infected with herpesvirus. 3. Relative amino acid content of various proteins formed after infection. *Virology* 1970; **40**: 90–101.

64. Bol S, Bunnik EM. Lysine supplementation is not effective for the prevention or treatment of feline herpesvirus 1 infection in cats: a systematic review. *BMC Veterinary Research* 2015; **11**: 284.

65. Cave NJ, Dennis K, Gopakumar G *et al.* Effects of physiologic concentrations of l-lysine on in vitro replication of feline herpesvirus 1. *American Journal of Veterinary Research* 2014; **75**: 572–580.

66. Stiles J, Townsend WM, Rogers QR *et al.* Effect of oral administration of L-lysine on conjunctivitis caused by feline herpesvirus in cats. *American Journal of Veterinary Research* 2002; **63**: 99–103.

67. Maggs DJ, Nasisse MP, Kass PH. Efficacy of oral supplementation with L-lysine in cats latently infected with feline herpesvirus. *American Journal of Veterinary Research* 2003; **64**: 37–42.

68. Rees TM, Lubinski JL. Oral supplementation with L-lysine did not prevent upper respiratory infection in a shelter population of cats. *Journal of Feline Medicine and Surgery* 2008; **10**: 510–513.

69. Fascetti AJ, Maggs DJ, Kanchuk ML *et al.* Excess dietary lysine does not cause lysine-arginine antagonism in adult cats. *Journal of Nutrition* 2004; **134**: 2042S–2045S.

70. Maggs DJ, Sykes JE, Clarke HE *et al.* Effects of dietary lysine supplementation in cats with enzootic upper respiratory disease. *Journal of Feline Medicine and Surgery* 2007; **9**: 97–108.

71. Drazenovich TL, Fascetti AJ, Westermeyer HD *et al.* Effects of dietary lysine supplementation on upper respiratory and ocular disease and detection of infectious organisms in cats within an animal shelter. *American Journal of Veterinary Research* 2009; **70**: 1391–1400.

72. Sandmeyer LS, Keller CB, Bienzle D. Effects of interferon-alpha on cytopathic changes and titers for feline herpesvirus-1 in primary cultures of feline corneal epithelial cells. *American Journal of Veterinary Research* 2005; **66**: 210–216.

73. Siebeck N, Hurley DJ, Garcia M *et al.* Effects of human recombinant alpha-2b interferon and feline recombinant omega interferon on in vitro replication of feline herpesvirus-1. *American Journal of Veterinary Research* 2006; **67**: 1406–1411.

74. Weiss RC. Synergistic antiviral activities of acyclovir and recombinant human leukocyte (alpha) interferon on feline herpesvirus replication. *American Journal of Veterinary Research* 1989; **50**: 1672–1677.

75. Cerruti RL, Connell EV, Trown PW *et al.* Synergistic interaction between interferon-alpha and acyclovir in the treatment of herpes simplex virus type 1 infection in mice. *Antiviral Research* 1985; Suppl 1: 217–223.

76. Haid C, Kaps S, Gonczi E *et al.* Pretreatment with feline interferon omega and the course of subsequent infection with feline herpesvirus in cats. *Veterinary Ophthalmology* 2007; **10**: 278–284.

77. Cocker FM, Howard PE, Harbour DA. Effect of human alpha-hybrid interferon on the course of feline viral rhinotracheitis. *The Veterinary Record* 1987; **120**: 391–393.

78. Ballin AC, Schulz B, Helps C *et al.* Limited efficacy of topical recombinant feline interferon-omega for treatment of cats with acute upper respiratory viral disease. *Veterinary Journal* 2014; **202**: 466–470.

79. Fenimore A, Carter K, Fankhauser J *et al.* Evaluation of intranasal vaccine administration and high-dose interferon-alpha 2b therapy for treatment of chronic upper respiratory tract infections in shelter cats. *Journal of Feline Medicine and Surgery* 2015; doi: 10.1177/1098612X15596199.

80. Slack JM, Stiles J, Leutenegger CM *et al.* Effects of topical ocular administration of high doses of human recombinant interferon alpha-2b and feline recombinant interferon omega on naturally occurring viral keratoconjunctivitis in cats. *American Journal of Veterinary Research* 2013; **74**: 281–289.

81. Gil S, Leal RO, Duarte A *et al.* Relevance of feline interferon omega for clinical improvement and reduction of concurrent viral excretion in retrovirus infected cats from a rescue shelter. *Research in Veterinary Science* 2013; **94**: 753–763.

82. Stiles J, Guptill-Yoran L, Moore GE *et al.* Effects of lambda-carrageenan on in vitro replication of feline herpesvirus and on experimentally induced herpetic conjunctivitis in cats. *Investigative Ophthalmology & Visual Science* 2008; **49**: 1496–1501.

83. Bagla VP, McGaw LJ, Eloff JN. The antiviral activity of six South African plants traditionally used against infections in ethnoveterinary medicine. *Veterinary Microbiology* 2012; **155**: 198–206.

84. Knight DA, Hejmanowski AQ, Dierksheide JE *et al.* Inhibition of herpes simplex virus type 1 by the experimental immuno-suppressive agent leflunomide. *Transplantation* 2001; **71**: 170–174.

85. Williams CR, Sykes JE, Mehl M *et al.* In vitro effects of the active metabolite of leflunomide, A77 1726, on feline herpesvirus-1. *American Journal of Veterinary Research* 2007; **68**: 1010–1015.

86. Beaumont SL, Maggs DJ, Clarke HE. Effects of bovine lactoferrin on in vitro replication of feline herpesvirus. *Veterinary Ophthalmology* 2003; **6**: 245–250.

87. Wilkes RP, Kania SA. Use of interfering RNAs targeted against feline herpesvirus 1 glycoprotein D for inhibition of feline herpesvirus 1 infection of feline kidney cells. *American Journal of Veterinary Research* 2009; **70**: 1018–1025.

88. Wilkes RP, Kania SA. Evaluation of the effects of small interfering RNAs on in vitro replication of feline herpesvirus-1. *American Journal of Veterinary Research* 2010; **71**: 655–663.

89. Wilkes RP, Ward DA, Newkirk KM *et al.* Evaluation of delivery agents used for introduction of small interfering RNAs into feline corneal cells. *American Journal of Veterinary Research* 2013; **74**: 243–247.

90. Lappin MR, Veir JK, Satyaraj E *et al.* Pilot study to evaluate the effect of oral supplementation of Enterococcus faecium SF68 on cats with latent feline herpesvirus 1. *Journal of Feline Medicine and Surgery* 2009; **11**: 650–654.

91. Lim CC, Reilly CM, Thomasy SM *et al.* 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* 2009; **70**: 394–403.

92. Cullen CL, Njaa BL, Grahn BH. Ulcerative keratitis associated with qualitative tear film abnormalities in cats. *Veterinary Ophthalmology* 1999; **2**: 197–204.

93. Sarisky RT, Quail MR, Clark PE *et al.* Characterization of herpes simplex viruses selected in culture for resistance to penciclovir or acyclovir. *Journal of Virology* 2001; **75**: 1761–1769.

94. Guardabassi L, Prescott JF. Antimicrobial stewardship in small animal veterinary practice: from theory to practice. *The Veterinary Clinics of North America. Small Animal Practice* 2015; **45**: 361–376, vii.

95. White ML, Chodosh J. Herpes simplex virus keratitis: a treatment guideline – 2014. *The American Academy of Ophthalmology Clinical Guidelines.* http://www.aao.org/clinical-statement/herpes-simplex-virus-keratitis-treatment-guideline#SUPPORTDOCUMENTATION [accessed on 6 March 2016].

96. De Vlaminck I, Khush KK, Strehl C *et al.* Temporal response of the human virome to immunosuppression and antiviral therapy. *Cell* 2013; **155**: 1178–1187.

97. Andrei G, Snoeck R, Goubau P *et al.* Comparative activity of various compounds against clinical strains of herpes simplex virus. *European Journal of Clinical Microbiology and Infectious Diseases* 1992; **11**: 143–151.

98. Babiuk LA, Meldrum B, Gupta VS *et al.* Comparison of the antiviral effects of 5-methoxymethyl-deoxyuridine with 5-iododeoxyuridine, cytosine arabinoside, and adenine arabinoside. *Antimicrobial Agents and Chemotherapy* 1975; **8**: 643–650.

99. Chen ZJ, Liu WG, Song JZ. [Experimental studies of 9-(1, 3-dihydroxy-2-propoxymethyl)-guanine(DHPG) against herpes simplex virus]. *Yao Xue Xue Bao* 1989; **24**: 331–334.

100. Boyd MR, Bacon TH, Sutton D *et al.* Antiherpesvirus activity of 9-(4-hydroxy-3-hydroxy-methylbut-1-yl)guanine (BRL 39123) in cell culture. *Antimicrobial Agents and Chemotherapy* 1987; **31**: 1238–1242.

101. De Clercq E, Sakuma T, Baba M *et al.* Antiviral activity of phosphonylmethoxyalkyl derivatives of purine and pyrimidines. *Antiviral Research* 1987; **8**: 261–272.

102. Gadler H, Larsson A, Solver E. Nucleic acid hybridization, a method to determine effects of antiviral compounds on herpes simplex virus type 1 DNA synthesis. *Antiviral Research* 1984; **4**: 63–70.

103. Ayisi NK, Gupta SV, Babiuk LA. Combination chemotherapy: interaction of 5-methoxymethyldeoxyuridine with trifluorothymidine, phosphonoformate and acycloguanosine against herpes simplex viruses. *Antiviral Research* 1985; **5**: 13–27.

104. Sandmeyer LS, Keller CB, Bienzle D. Effects of cidofovir on cell death and replication of feline herpesvirus-1 in cultured feline corneal epithelial cells. *American Journal of Veterinary Research* 2005; **66**: 217–222.

105. Bronson JJ, Ghazzouli I, Hitchcock MJ *et al.* Synthesis and antiviral activity of the nucleotide analogue (S)-1-[3-hydroxy-2-(phosphonylmethoxy)propyl]cytosine. *Journal of Medicinal Chemistry* 1989; **32**: 1457–1463.

106. Bestman-Smith J, Boivin G. Herpes simplex virus isolates with reduced adefovir susceptibility selected in vivo by foscarnet therapy. *Journal of Medical Virology* 2002; **67**: 88–91.

107. Collins P. The spectrum of antiviral activities of acyclovir in vitro and in vivo. *Journal of Antimicrobial Chemotherapy* 1983; **12**(Suppl B): 19–27.