

Efficacy of antiviral agents in feline herpetic keratitis: Results of an *in vitro* study

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Abstract

Purpose. To determine, by a plaque reduction assay, the *in vitro* efficacy of novel antiviral agents in the treatment of feline herpes virus 1 (FHV-1) keratitis in the domestic cat (*Felis felis*).

Materials and methods. A standard plaque reduction assay was performed using a laboratory strain of FHV-1 and embryo-derived feline kidney cells to determine the *in vitro* efficacy of the antiviral drugs penciclovir (PCV), bromovinyldeoxyuridine (BVdU), and (*S*)-9-(3-hydroxy-2-phosphonylmethoxypropyl) adenine (HPMPA) and to compare these with the drugs acyclovir (ACV) and trifluorothymidine (TFT). Efficacy was assessed by determining the dose of drug at which 50% plaque reduction was noted (ED_{50}).

Results. HPMPA was found to have greatest antiviral activity (ED_{50} 0.07 μ g/ml). ACV was least active (ED_{50} 24 μ g/ml), while TFT was active with an ED_{50} of 5.7 μ g/ml. PCV and BVdU had intermediate activity (ED_{50} 1.6 and 1.7 μ g/ml, respectively).

Conclusions. This study suggests that the efficacy of HPMPA, BVdU, and penciclovir in cats with herpesviral keratitis should be determined *in vivo* as their efficacy *in vitro* was substantially greater than that of acyclovir, already shown to have demonstrable but limited clinical antiviral activity.

Keywords: cat; feline; herpes virus; cornea; treatment; acyclovir; antiviral

Introduction

The treatment of feline herpes virus (FHV-1) keratitis and keratoconjunctivitis is complicated in the United Kingdom by lack of efficacious licensed topical antiviral medications. Idoxuridine and vidarabine are widely used in the United States but are unavailable in the United Kingdom. Topical trifluorothymidine (TFT) is effective but not available through standard pharmaceutical wholesalers. Acyclovir (ACV) is a widely available antiviral agent used for treatment of herpes simplex virus (HSV) infections in man including herpetic disease of the ocular surface, but though it has some activity against FHV-1, this is only seen clinically with treatment five times daily.¹ In this study, we seek to investigate the effects of novel antiviral agents on replication of FHV-1 *in vitro* and compare these with the activity of ACV and TFT with the aim of developing drugs that will be efficacious in the treatment of FHV-1 keratitis.

Feline herpesvirus is a pathogen causing significant morbidity in the cat population, and ocular lesions associated with the virus play a significant part in the pathology of viral infection.² Ocular lesions associated with FHV-1 include conjunctivitis, dendritic and geographic corneal ulceration, and vascular keratitis together with the less familiar pathologies of corneal sequestrum and eosinophilic keratitis, also reported sometimes to be associated with FHV-1 infection. Treatment for ocular FHV-1 infection is problematic in the United Kingdom given the poor availability of antiviral agents as noted above. The work of Nasisse and colleagues³ showed that, with regard to *in vitro* efficacy of antiviral agents studied, TFT had a much greater efficacy than

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idoxuridine, for which efficacy was greater than BVdU, and that all these drugs had a much greater efficacy than did ACV. The authors concluded that ACV was of no value in feline herpetic keratitis. Similar conclusions had been reached by previous studies both *in vitro*^{4,5} and *in vivo*.^{6,7} Yet we have shown that topical ACV does have a significant if limited effect on FHV-1 lesions in the eye.¹ In that study, we showed, through *in vivo* and *in vitro* investigation of antiviral effects of ACV that the drug levels produced at the ocular surface by topical application of a 3% ointment were sufficient for antiviral activity of the drug if used five times daily. More recently produced antiviral medications (penciclovir [PCV], bromovinyldeoxyuridine [BVdU], and [S]-9-[3-hydroxy-2-phosphonylmethoxypropyl] adenine [HPMPA]) have a greater efficacy against HSV than does ACV, and here we ask whether that improved activity is also the case with FHV-1.

Materials and methods

Feline embryo-derived cells were cultured to confluence in 24-well culture plates using Eagle's minimum essential medium (EMEM) supplemented with 5% fetal calf serum (FCS) and antibiotic and antifungal agents. To each well an overlay of EMEM supplemented with 1% FCS, antibiotic agents, antifungal agents, and carboxymethylcellulose as a thickening agent was added together with the antiviral agent to be studied at concentrations as documented in Table 1. Antiviral agents were initially prepared by dilution of dry powder in phosphate buffered saline to a concentration of 1mg/ml. Drug was subsequently serially diluted to give concentration ranges as detailed in table 1. Plates were seeded with a laboratory strain of FHV-1 (B927) at a viral load sufficient to provide approximately 100PFU per well, as previously determined empirically. All plates were incubated for 30 hr at 37°C, after which they were stained using crystal violet and the cytopathic effects observed using light microscopy to identify plaques of cell death. Drug efficacy

Table 1. Concentrations of drugs used, concentration at which 50% of maximum plaque reduction was reached (ED₅₀), and concentration at which 90% of maximum plaque reduction was reached (ED₉₀).

Drug	Concentration range tested (µg/ml)	ED ₅₀ (µg/ml)	ED ₉₀ (µg/ml)
ACV	0.001-50	24	50
TFT	0.001-30	5.7	16.8
PCV	0.001-10	1.6	7.8
BVdU	0.001-10	1.7	7.8
HPMPA	0.001-10	0.07	1.4

ACV, acyclovir; TFT, trifluorothymidine; PCV, penciclovir; BVdU, bromovinyldeoxyuridine; HPMPA, (S)-9-(3-hydroxy-2-phosphonylmethoxypropyl) adenine.

was determined by documenting the reduction in number of viral plaques observed. All assays were repeated in triplicate with data evaluated being the mean ± standard deviation of these replicate experiments. Plaque reduction was plotted for varying concentrations of the drugs and thus the ED₅₀ and ED₉₀, that is to say the drug concentrations at which plaque numbers were reduced by 50% and 90%, respectively, were determined by graphical interpolation.

Results

Results of the plaque reduction assay described above are shown graphically in Figure 1, and ED₅₀ and ED₉₀ values shown in Table 1. HPMPA was found to have most activity, ACV was least active, and TFT, PCV, and BVdU had intermediate activity.

Discussion

De Clercq has rightly stated that "antiviral chemotherapy came of age with the advent in 1977 of acyclovir as the first truly specific antiviral agent."⁸ ACV and its structural analogs PCV and ganciclovir have a broad spectrum of activity against HSV-1 and 2 and, in the case of the latter drug, cytomegalovirus also. Their viral specificity relies on the requirement for phosphorylation of the prodrug by viral thymidine kinase (TK). It is, however, here that ACV and its analogs have their Achilles' heel as far as action against other herpesviruses is concerned. ACV is not as active against all herpesviruses as it is against HSV-1. The *in vitro* results of this study confirm those of previous workers^{2,4,7} that ACV has a poor activity against FHV-1. Importantly however, the *in vitro* results presented here show both an ED₅₀ and ED₉₀ substantially greater than that anticipated at the ocular surface during the topical application of 3% acyclovir ointment, that being 12 mM. This finding explains the apparent discrepancy between previous reports from the United States of the lack of efficacy of ACV and our report of its ameliorative effects in herpetic keratitis.¹ For researchers in the United States, with only the orally administered drug available in the USA, the systemic toxicity of ACV when given at doses high enough to reach clinically effective concentrations at the ocular surface rendered acyclovir ineffective for the treatment of ocular herpetic keratitis in the cat. When available as a topical ocular preparation, as in the United Kingdom, the drug concentration at the ocular surface is efficacious in its virustatic effect. Thus, the *in vitro* results presented here demonstrate that ACV may indeed have some efficacy when applied frequently. The question remains, however, as to the reason for the apparent lack of efficacy of ACV when compared to the other drugs studied here. Given that ACV is a prodrug that requires phosphorylation before it has antiviral efficacy, viruses resistant to ACV may lack a functional viral TK or more likely have a viral TK with a poor affinity for ACV. It may, however, be a change in the

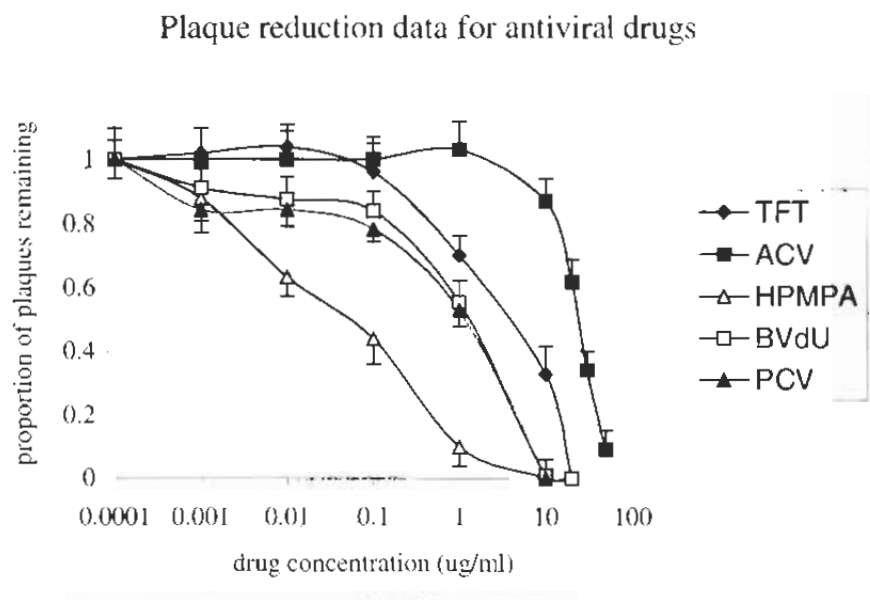


Figure 1. Graphical representation of plaque reduction data for the five antiviral drugs tested *in vitro* for efficacy against feline herpesvirus.

viral DNA polymerase upon which the active compound acts that accounts for resistance.⁸ These two possibilities have been found to account for resistance to ACV in clinical drug resistance in HSV-1 isolates and thus could conceivably be the reason for reduced efficacy of ACV in FHV-1.⁹ TK-deficient mutant FHV-1 is attenuated in its clinical effects¹⁰ and thus wild-type FHV-1 is TK-competent. Given the wide divergence in gene sequence between FHV-1 and other alpha herpesviruses,¹¹ it is perhaps not surprising that the FHV-1 TK should not show great affinity for or phosphorylating activity against ACV. PCV has a similar activity spectrum and mechanisms of action to ACV, but its higher potency might explain why in this study it overcomes the relative ineffective TK activity of FHV-1 against ACV.

The viral specificity of BVdU relies on the 2(E)-bromovinyl group and, after phosphorylation by viral TK, it can act either as a competitive inhibitor of viral DNA polymerase or as an alternative substrate. BVdU is effective against HSV-1 but not HSV-2, as the TK of the latter virus is unable to phosphorylate BVdU. It is, however, efficacious in infections with swine herpesvirus type 1, bovine herpesvirus type 1, simian varicella virus, and herpesvirus platyrrhinae but not equine herpesvirus type 1, again because of a lack of phosphorylation of the drug.¹² The results of this study suggest that BVdU would be efficacious in FHV-1 infections, but it is here not possible to assess whether this is because the FHV-1 TK is active against BVdU or whether, as with PCV, it is likely to be the greater antiviral action that overcomes the poor phosphorylating effects of FHV-1 TK against the drug.

HPMPA was produced as a hybrid molecule combining the reactive features of an acyclic nucleoside analog such as (S)-9-(2,3-dihydroxypropyl)adenine first produced in

Czechoslovakia in the 1970s¹³ together with those of a phosphonate analog such as (phosphonylmethoxypropyl)adenine (Fosgarnet).¹⁴ It has activity against a wide spectrum of DNA viruses including the herpesviruses and does not rely on viral TK for activation. This may explain its activity against FHV-1 in the current study.

Conclusions

Our study thus shows that other antiviral agents hold much promise for more effective treatment of disease associated with ocular surface infection with FHV-1. HPMPA is highly effective against FHV-1, whereas BVdU and PCV, the latter of which is available in the United Kingdom as a topical treatment for dermatological use, have a less pronounced, but still clinically relevant effect but nevertheless one substantially greater than that of ACV. These results suggest that HPMPA, BVdU, and PCV should be considered for development as ophthalmic agents for the treatment of feline herpetic keratitis. We are currently developing a trial of PCV for ocular herpetic disease in the cat.

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