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Penciclovir is a potent inhibitor of feline herpesvirus-1 with susceptibility determined at the level of virus-encoded thymidine kinase

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Abstract

Feline herpesvirus-1 (FHV-1) is the causative agent of a severe ocular disease in cats for which a safe potent antiviral chemotherapeutic agent is highly demanded. The sensitivity of FHV-1 to inhibition by three anti-herpetic nucleoside analogues [acyclovir (ACV), penciclovir (PCV) and cidofovir (CDV)] was tested by means of yield reduction assay. ACV showed very poor ability to inhibit FHV-1 replication. At low multiplicity of infection (MOI), both PCV and CDV were nearly equally effective with IC_{50} values ranging between 6 and 8 μg/ml. However, when the MOI was raised to 3 PFU/cell, the activity of CDV was markedly reduced (IC_{50} 25 μg/ml), while that of PCV remained relatively low (IC_{50} 10 μg/ml). Although FHV-1 is normally insensitive to ACV, it exhibited >1000-fold increase in sensitivity when the thymidine kinase (TK) encoded by herpes simplex virus-1 (HSV-1) was supplied in trans. Furthermore, three PCV-resistant FHV-1 variants selected *in vitro* were shown to carry mutations in the TK gene. Taken together, these data provided direct evidence that PCV is a potent selective inhibitor of FHV-1 and that the virus-encoded TK is an important determinant of the virus susceptibility to nucleoside analogues.

Keywords: Feline herpesvirus; Thymidine kinase; Penciclovir

1. Introduction

Feline herpesvirus-1 (FHV-1), a member of the α-herpesvirus subfamily, is the most common viral pathogen of domestic cats worldwide. It causes a severe upper respiratory tract and ocular disease in cats characterized by conjunctivitis, profuse ocular and nasal discharges, and in some cases, severe keratitis and corneal ulceration. In kittens, the infection can generalize resulting in mortality rates of up to 50% (Gaskell and Dawson, 1994; Andrew, 2001; Maggs, 2005). Despite routine vaccination, FHV-1 disease continues to pose a major threat to feline health, due to the establishment of lifelong neuronal latency interspersed with episodes of viral reactivation and shedding (Gaskell and Willoughby, 1999; Stiles, 2003).

Antiviral chemotherapy is a standard practice in the management of herpesvirus infections in humans, and currently there are 11 licensed anti-herpetic compounds available in the human clinic (De Clercq et al., 2006). Two important groups of anti-herpetic drugs, which target the viral DNA polymerase, are the acyclic nucleoside analogues [e.g. acyclovir (ACV), penciclovir (PCV) and ganciclovir (GCV)] and acyclic nucleoside phosphonates [e.g. cidofovir (CDV)] (De Clercq and Holý, 2005; Field and Whitley, 2005). Herpesvirus-encoded thymidine kinase (TK) is the molecular basis for the selective activity of acyclic nucleoside analogues. Studies on the mode of action of ACV have shown that it is preferentially phosphorylated in infected cells by the viral TK to ACV-monophosphate (ACV-MP), which is in turn converted to ACV-triphosphate (ACV-TP) by cellular kinases. ACV-TP is the active form that inhibits herpesvirus DNA replication (Elion, 1993; Coen and Schaffer, 2003). Since the acyclic nucleoside phosphonates already contain a phosphonate group, they need only two, instead of three, phosphorylation steps in order to reach the active form, thus they do not depend on the viral TK to exert their antiviral action (De Clercq, 2003).

No effective antiviral therapy for the treatment of FHV-1 infections currently exists. Acyclovir, a potent inhibitor of herpes simplex virus-1 (HSV-1) with an exceptional safety record (Elion, 1993), is the only anti-herpetic drug that has so far received adequate clinical and research attention in veterinary

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medicine. The similarities between the ocular diseases caused by FHV-1 and HSV-1 have encouraged the use of ACV to treat FHV-1 infections in cats. However, ACV demonstrated relatively poor efficacy against FHV-1 (Collins, 1983; Nasisse et al., 1989). Moreover, poor bioavailability and toxicity for cats of valaciclovir, the oral prodrug of ACV, limited further clinical use of this drug in cats (Owens et al., 1996; Nasisse et al., 1997). Therefore, there is a distinct need for an alternative therapeutic antiviral agent for treating cats with recurrent FHV-1 disease. In order to achieve a good degree of compliance from cat owners, an effective antiviral drug that could be administered infrequently to cats would be greatly beneficial. From this point of view, both PCV and CDV appear to be attractive candidates because they share the property of conferring a relatively long-lasting antiviral action due to the long half-life of their metabolites formed intracellularly after drug uptake by the cells (Vere Hodge and Perkins, 1989; Earnshaw et al., 1992; Neyts et al., 1991; Ho et al., 1992).

The activity of antiviral agents can be measured by different methods. Plaque reduction assay (PRA), which measures the ability of an antiviral compound to inhibit the virus-induced cytopathic effect (CPE), is the most widely used method for routine antiviral susceptibility testing (Swierkosz and Biron, 1995; Lurain and Thompson, 1997). Yield reduction assay (YRA) is a more stringent alternative method that measures the ability of a certain drug to reduce the total yield of infectious virus. This assay is considered to be more clinically relevant because it reflects virus shedding in natural infections (Bacon and Schinazi, 1993; Boyd et al., 1993). Although several investigators studied the *in vitro* susceptibility of FHV-1 to anti-herpetic compounds, to date all these have relied solely for their assessment on PRA, and none attempted to examine the activity of those compounds using YRA. One PRA-based study has demonstrated PCV and CDV to be nearly equipotent against FHV-1 (Maggs and Clarke, 2004). One of the objectives of the work described in this report was to compare the anti-FHV-1 activity of PCV and CDV at several multiplicities of infection (MOIs) by YRA. PCV displayed a superior anti-FHV-1 activity over a wider range of MOIs. Since PCV is a TK-dependent drug, our attention was directed toward investigating the role of TK-mediated phosphorylation in determining the susceptibility of FHV-1 to nucleoside analogues. Two approaches were taken to address this point. Firstly, we hypothesized that the observed insensitivity of FHV-1 to ACV is due to inefficient phosphorylation by the FHV-1 encoded TK. HSV-1 TK is known to phosphorylate ACV, albeit at a relatively low efficiency, the levels of ACV-MP produced are sufficient to inhibit the viral DNA polymerase after being converted to the active triphosphate form (Griffiths, 1994). A permanently-transfected CRFK cell line expressing the HSV-1 TK gene was produced. The hypothesis described above was tested by examining the sensitivity of FHV-1 to a panel of TK-dependent compounds (ACV, PCV, GCV) and a positive control TK-independent compound (CDV) in the HSV-1 TK-transformed versus negative control (transfected with an empty plasmid) CRFK cells. Secondly, we selected FHV-1 PCV-resistant variants; this resistant phenotype was associated with mutations in the virus-encoded TK as described below.

2. Materials and methods

2.1. Viruses, cells and antiviral compounds

Two laboratory strains, HSV-1 (SC16) and FHV-1 (B927), were used. The later virus and the Crandell–Reese Feline Kidney (CRFK) cells were kindly supplied by Prof. R. Gaskell, University of Liverpool, UK. CRFK cells were maintained in Dulbecco's modified Eagle's medium (DMEM) as previously described (Williams et al., 2004). Acyclovir and ganciclovir were purchased from Sigma. Cidofovir was a gift from Prof. E. De Clercq, Rega Institute of Medical Research, Belgium. Penciclovir was a gift from Novartis pharmaceuticals, Switzerland.

2.2. Cloning and plasmid construction

HSV-1 (SC16) genomic DNA was extracted using High Pure Viral Nucleic Acid kit (Roche). The entire HSV-1 and FHV-1 TK genes were PCR-cloned using PWO high-fidelity DNA polymerase (Roche). The primers (5′-GAAGGATCCACCATGGCTTCGTACCCCGGCCAT-3′ and 5′-GTTGAATTCTCAGTTAGCCTCCCCCATCTC-3′) were designed with overhanging BamHI and EcoRI restriction sites at their 5′ ends. The recombinant plasmid pcHS16TK was obtained by directional cloning of the HSV-1 TK gene into the mammalian expression vector pcDNA3 using standard techniques (Sambrook and Russell, 2001).

2.3. FHV-1 yield reduction assay (YRA)

Confluent monolayers of CRFK cells, prepared in 24-well tissue culture plates, were infected with FHV-1 at three different MOIs (0.1, 3 and 10 PFU/cell). After the virus was allowed to adsorb for 45 min at 37 °C, the inoculum was aspirated, and the infected cell monolayers were washed twice with warm DMEM. The infected monolayers were overlaid with DMEM (1 ml/well) containing an increasing concentration (0.001, 0.01, 0.1, 1, 10 or 50 μg/ml) of each test compound (ACV, PCV and CDV) in triplicate wells. Drug-free medium was added to control wells. After 48 h of incubation at 37 °C in a humidified CO_2 incubator, culture supernatants were harvested and the amount of infectious virus was titrated on fresh monolayers of CRFK cells by standard PRA as described previously (Williams et al., 2004). The virus yield was plotted as the % of untreated control against the $\log_{10}$ of drug concentration, and the concentration of drug that resulted in 50% reduction in the virus yield (IC_{50}) was determined from the graph.

2.4. Production of permanently-transfected CRFK cell line expressing HSV-1 TK

Twenty-five micrograms of the empty pcDNA3 (negative control) or the pcHS16TK plasmids were used to transfect CRFK cells by the calcium phosphate precipitation method as previously described (Graham and van der Eb, 1973). A clone of the transformed cells was selected using DMEM containing 400 μg/ml of G418 (Sigma). The HSV-1 TK polypeptide

Table 1
The anti-FHV-1 (B927) activity of ACV, PCV and CDV as measured by YRA at three levels of MOI (0.1, 3 and 10 PFU/cell)

MOI	Mean $IC_{50} \pm$ S.D.					
	ACV		PCV		CDV	
	μg/ml	μM	μg/ml	μM	μg/ml	μM
0.1	38 ± 1.75	168 ± 7.77	7.8 ± 0.13	30.8 ± 0.50	5.6 ± 0.25	18.8 ± 0.84
3	50 ± 1.15	222 ± 5.00	10 ± 1.00	39.5 ± 4.00	25 ± 0.89	85.0 ± 2.99
10	>50	>222	33 ± 1.18	130 ± 4.66	50 ± 5.38	168 ± 18.0

Results are presented as the mean IC_{50} values obtained from three independent experiments carried out in triplicate ±S.D.

was detected in whole cell lysates by western blot using mouse anti-HSV-1 TK monoclonal antibody (kindly supplied by Prof. William Summers, Yale University, USA).

2.5. Selection and characterization of the PCV-resistant FHV-1 mutants

A three times plaque-purified stock of the FHV-1 (B927) was obtained as previously described (Andrei et al., 2001). A confluent CRFK monolayer, prepared in 175-cm^2 tissue culture flask, was infected with wild-type FHV-1 B927 at an MOI of 0.01 PFU/cell. After the virus was allowed to adsorb for 45 min, the infected monolayer was overlaid with DMEM containing 2.5 μg/ml of PCV. When the culture demonstrated complete CPE, typically after 2 days, the cell sheet was detached and subjected to three cycles of freeze–thawing. The supernatant was clarified by centrifugation and used to infect fresh CRFK cells. This procedure was repeated three times with doubling the PCV concentration with every passage, i.e. 5, 10 and 20 μg/ml. Three plaques, which were detectable at 20 μg/ml of PCV, were isolated and finally passaged in drug-free DMEM. The obtained virus stock was titrated and subsequently used for drug-susceptibility testing. The viral genomic DNA was extracted and the TK genes of the three PCV-resistant mutants were subjected to DNA sequencing which was conducted in the Biochemistry department, Cambridge University, using an Applied Biosystems 3730xl DNA sequencer.

3. Results

3.1. Comparing the anti-FHV-1 activity of PCV and CDV by YRA

Since it is likely that during a clinical infection, individual cells will be infected with a variety of MOIs, it was important to compare the activity of the tested anti-herpetic compounds at several MOIs of FHV-1. A genuinely superior compound would be expected to have better activity under most, if not all, MOI conditions (Littler, 1994). As shown (Table 1), ACV was demonstrated to be inefficient in inhibiting FHV-1 replication at all MOI levels tested. At low MOI (0.1 PFU/cell), both PCV and CDV were very effective with IC_{50} values ranging between 6 and 8 μg/ml. However, when the MOI was raised to 3 PFU/cell (at which approximately 95% of cells would be infected with at lease one virus particle), the activity of CDV was markedly reduced with an IC_{50} of 25 μg/ml, while that of PCV remained relatively low (10 μg/ml). The activity of the three compounds was reduced at the highest level of MOI tested (10 PFU/cell).

3.2. Antiviral susceptibility of FHV-1 in HSV-1 TK-transformed cells

A permanently-transfected CRFK cell line expressing the HSV-1 TK gene was produced. The HSV-1 TK polypeptide expressed within the pcHS16TK CRFK cells was detected by Western blotting as two bands having molecular weights of approximately 41 and 39 kDa (data not shown); this is believed to be due to internal initiation of translation which occurs with approximately 10% of the TK mRNA (Marsden et al., 1983; Haarr et al., 1985). The antiviral activity of a group of TK-mediated antiviral compounds (ACV, PCV and GCV) and a TK-independent drug (CDV) against FHV-1 (100 PFU/well of a standard six-well plate) were compared in pSC16TK versus negative control pcDNA3 CRFK cells using standard PRA as previously described (Williams et al., 2004). As shown (Table 2), supplying HSV-1 TK in trans resulted in a marked increase (>1000-fold) in the sensitivity to ACV. Also, a smaller but significant increase was also observed for PCV and GCV (20- and 240-fold, respectively). As expected, the drug with a TK-independent mechanism of action (CDV), showed no change in sensitivity.

3.3. Antiviral sensitivity and cross-resistance of the PCV-resistant FHV-1 mutants

Three PCV-resistant mutants, named PrD, PrE, and PrG, were independently selected from a parental three times plaque-purified virus preparation of FHV-1 B927, by four serial passages of the virus in CRFK cells in the presence of increasing

Table 2
Comparative antiviral susceptibility of FHV-1 in pcSC16TK versus negative control pcDNA3 CRFK cells by PRA

Drug	Mean IC_{50} (μM) ± S.D.		Fold of change
	pcDNA3 CRFK cells	pcSC16TK CRFK cells	
ACV	110 ± 14.38	0.1 ± 0.006	1100
PCV	24.5 ± 3.66	1.25 ± 0.26	20
GCV	12 ± 1.65	0.05 ± 0.004	240
CDV	24.8 ± 3.15	24.8 ± 4.5	1

Results are presented as the mean values of three independent experiments ±S.D.

concentrations of PCV (2.5, 5, 10 and 20 μg/ml). The susceptibility profiles of the three selected FHV-1 mutants to PCV, GCV, ACV, and CDV were determined by PRA in CRFK cells (Table 3). All three mutants selected with PCV showed a 7–12-fold increase in the IC_{50} value of PCV over that of the wild-type (WT) parental virus; they were also cross-resistant to GCV but remained sensitive to CDV. Interestingly, there was only a 1.5-fold increase in the IC_{50} value of ACV when the mutants were compared with the WT. In order to investigate whether the mechanism of resistance was due to a mutation of the TK or DNA pol genes, the susceptibility of the three mutants to PCV was determined by PRA in the HSV-1 TK-transformed CRFK cells. All three mutants regained their sensitivity to PCV (data not shown), which suggested that the resistance was due to mutations of the FHV-1 TK.

3.4. DNA sequence analysis of the TK gene from PCV-resistant mutants

The FHV-1 TK coding region is 1029 bp in length and encodes a protein of 343 amino acids (Nunberg et al., 1989). The TK genes of the plaque-purified WT FHV-1 B927 and the three selected mutants were subjected to DNA sequencing. Alignment of the final sequences revealed no differences between the parental WT plaque-purified FHV-1 B927 and the published sequence of the FHV-1 TK gene (GenBank accession number M26660), however, three distinct changes were detected with the PCV-resistant mutants. The mutant PrG had a substitution (C → T) at nt 241, resulting in an early termination codon (TGA). The mutant PrE had a double deletion of A348 and G349, resulting in a frame-shift. The predicted TK polypeptide produced by these two mutants was truncated; suggesting that the enzyme produced was likely to be completely inactive. In the case of mutant PrD, a triple deletion of ATT (nt 789–791) was detected; this deletion resulted in the loss of one amino acid (Leu-263) without changing the reading frame. The deleted Leu-263 was a part of the DTLF motif which is conserved among all α-herpesvirus TKs (Balasubramaniam et al., 1990).

4. Discussion

The main findings of this paper are: (i) PCV exhibited a more potent specific anti-FHV-1 activity than CDV. (ii) Inefficient phosphorylation of ACV by the FHV-1 encoded TK was responsible for the observed *in vitro* insensitivity of this virus to the antiviral action of ACV. (iii) The FHV-1-encoded TK contributes significantly to the differential antiviral activity of the nucleoside analogues.

Although the list of viruses infecting domestic animals is continuously expanding, the veterinary use of antiviral drugs is relatively uncommon. Perhaps the most frequently reported use of antiviral therapy in veterinary medicine is for the treatment of FHV-1-related ophthalmic disease. This has been partly due to the many similarities that exist between the pathogenesis of HSV-1 in humans and FHV-1 in cats (Stiles, 2003; Galle, 2004).

PCV and CDV were previously reported to have nearly equivalent anti-FHV-1 effects by PRA (Maggs and Clarke, 2004).

Table 3
Susceptibility profiles of the FHV-1 PCV-resistant mutants

FHV-1 strain	PCV		GCV		ACV		CDV	
	IC_{50} (μg/ml) ± S.D.	Fold resistance	IC_{50} (μg/ml) ± S.D.	Fold resistance	IC_{50} (μg/ml) ± S.D.	Fold resistance	IC_{50} (μg/ml) ± S.D.	Fold resistance
WT	3 ± 0.26	NA[a]	1.90 ± 0.35	NA[a]	19.95 ± 0.92	NA[a]	3.2 ± 0.36	NA[a]
PrD	30 ± 3.50	10	38.5 ± 6.39	20	31.5 ± 1.24	1.5	3.0 ± 0.45	0.9
PrE	19 ± 2.02	6	40.5 ± 1.54	21	30.0 ± 2.29	1.5	3.0 ± 0.38	0.9
PrG	38 ± 3.78	12	31.5 ± 3.56	16.5	29.5 ± 1.56	1.5	2.7 ± 0.22	0.85

Results are presented as the mean values of three independent experiments ±S.D.
[a] Not applicable.

Both drugs have potent antiviral effects *in vitro* and *in vivo* against several herpesviruses of human and veterinary importance (De Clercq and Holý, 1991; Gibson et al., 1992; Boyd et al., 1993; Gilliam and Field, 1993). The YRA data presented here have confirmed previous findings that FHV-1 is relatively insensitive to ACV. PCV retained its inhibitory effect on FHV-1 replication over a wider range of MOIs than CDV, which seemed to be highly sensitive to any increase in the amount of input virus (Table 1). The IC_{50} of PCV remained relatively low (7–10 μg/ml) over the range of 0.1–3 MOI, reaching up to 33 μg/ml at the highest MOI tested (10 PFU/cell). CDV was highly effective at the lowest MOI tested (0.1 PFU/cell) with an IC_{50} value of 5 μg/ml; however, when the MOI was raised, the efficacy of the drug dropped markedly with an IC_{50} value of 25 μg/ml at MOI of 3 and 50 μg/ml at MOI of 10. An inverse relationship between the initial MOI and the potency of ACV and PCV against HSV-1 has been previously reported (Harmenberg et al., 1980; Bacon and Schinazi, 1993), therefore, the observed reduction in activity at the highest MOI is not surprising. However, the significant reduction of CDV's potency in the YRA compared to that in the PRA suggested that this compound has a MOI-dependent anti-FHV-1 activity. These findings have also highlighted some important limitations associated with the PRA, which is considered by many investigators as the "gold standard" measurement of the activity of antiviral drugs. The major advantage of PRA is its overall simplicity. Nonetheless, because of the need to have a countable number of plaques, this assay can only be performed at very low MOI (approximately 50 PFU/well), which could be misleading sometimes (Swierkosz and Biron, 1995; Boivin, 1998).

By plaque reduction assay, the IC_{50} values of ACV for HSV-1 and FHV-1 were 0.1 μM (0.022 μg/ml) and 140 μM (31 μg/ml), respectively; this indicated about a 1400-fold decrease in sensitivity of FHV-1 to ACV as compared with HSV-1 (Collins, 1983). Several factors could account for the relative insensitivity of FHV-1 to the antiviral action of ACV, including: (i) poor uptake of ACV by feline cells, (ii) inefficient phosphorylation by the virus-encoded TK, (iii) failure of the feline cellular kinases to convert the monophosphate form of the drug into its triphosphate active form, (iv) rapid turnover of ACV-TP inside infected feline cells, or (v) inefficient inhibition of the viral DNA polymerase by the active form of the drug. The marked reduction in the IC_{50} value of ACV (Table 2) against FHV-1 (from 110 to 0.1 μM) when HSV-1 TK was supplied in trans by means of a transformed feline cell line provided direct evidence that FHV-1 was sensitive to the tri-phosphorylated form of ACV, and that inefficient phosphorylation of ACV by the FHV-1 encoded TK was responsible for the observed *in vitro* insensitivity of this virus to the antiviral action of ACV. It can be safely assumed that FHV-1 DNA polymerase has a high affinity for the ACV-TP, and, hence explains the observed inhibitory effect on virus replication.

Studies of herpesvirus drug resistance have helped to formulate a better understanding of the molecular mechanisms of antiviral action, viral enzyme structure-function relationships, and clinical drug resistance (Field, 2001; Gilbert et al., 2002). Three mutants, designated PrE, PrD, and PrG, were randomly selected, and tested for their sensitivity towards PCV, GCV, ACV, and CDV. All three mutants selected with PCV were cross-resistant to GCV and ACV, but remained sensitive to CDV (Table 3). ACV, PCV and GCV all rely on viral TK expression for their initial phosphorylation. Therefore, TK-negative strains are generally resistant to these compounds, yet remain sensitive to CDV, which inhibits viral DNA replication independently of the viral TK (Morfin and Thouvenot, 2003). Interestingly, in contrast to the marked shift in the IC_{50} values for PCV and GCV (6–12 and 15–20-fold, respectively), only a 1.5-fold increase was detected for the IC_{50} value of ACV as compared with the WT (Table 3). This result suggested that the initial activation of ACV inside FHV-1-infected cells may be largely due to non-specific TK-independent phosphorylation mechanisms. A trace of ACV phosphorylation was previously reported to occur in uninfected Vero cells, which was later attributed to the activity of the cytoplasmic enzyme 5′-nucleotidase (Furman et al., 1981; Keller et al., 1985). Therefore, a possible explanation is that ACV could be non-specifically phosphorylated by feline cellular kinases and that the minimal levels of tri-phosphorylated ACV may be sufficient to inactivate the viral DNA polymerase, which, in turn, could be responsible for the weak inhibitory action of ACV against FHV-1.

Sequencing of the TK gene of the FHV-1 mutants revealed three distinct changes which included a substitution and two deletions. The majority of ACV-resistant HSV-1 clinical isolates contained frame-shift mutations within two long homopolymeric nucleotide runs (G7 and C6) which were considered as hot spots for mutations within the HSV-1 TK gene (Sasadeusz et al., 1997). However, in the present study, the FHV-1 TK mutations were not highly localised to the G/C-rich regions. Notably, FHV-1 TK gene contains fewer homopolymer repeats, and overall has a lower G/C content (44%) than HSV-1 (65%). Resistant mutants of FHV-1 were readily selected from a homogeneous plaque-purified stock after three successive passages in the presence of high concentrations of PCV. The relatively low fidelity of herpesvirus DNA replication is thought to be responsible for this naturally occurring variation (Knopf, 1979; Hall and Almy, 1982). Two changes in mutants PrE and PrG had resulted in either early termination or frame-shift, and consequently the produced TK polypeptides are predicted to be inactive. The PrD mutant had a triple deletion of ATT which resulted in loss of a Leu-263, without changing the reading frame. This mutation was particularly interesting because it was located within a conserved motif (DTLF) among all α-herpesviruses. The exact function of this motif has not yet been fully elucidated (Balasubramaniam et al., 1990), and only one substitution mutation affecting this motif has been reported in the literature for HSV-2, where Thr-287 was replaced by Met leading to a TK-negative phenotype (Gaudreau et al., 1998). The fact that this TK mutation has also resulted in a resistant FHV-1 phenotype, suggested that the DTLF motif was essential for enzyme activity.

To conclude, antiviral agents are often ranked according to their performance in PRA; however, this always has to be backed up with a more stringent assessment using YRA. Whereas FHV-1 is normally relatively insensitive to ACV, it acquired sensitivity in the TK-transformed cells, in which the resident HSV-1 TK

was able to phosphorylate the drug. PCV is generally considered as a safe drug; clinical trials in man showed that famciclovir, the oral form of PCV, is well tolerated by patients with either herpes zoster or genital herpes (Saltzman et al., 1994). Further trials to test the safety and efficacy of PCV in cats seem to be warranted.

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