

Efficacy of topical aciclovir for the treatment of feline herpetic keratitis: results of a prospective clinical trial and data from in vitro investigations

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This study aimed to evaluate the efficacy of topical ophthalmic aciclovir applied five times daily as a treatment for feline herpesvirus type 1 (FHV-1) keratitis in a group of cats in a first-opinion practice setting. Cats with ocular signs indicative of FHV-1 or *Chlamydophila* species infection, predominantly conjunctivitis and keratitis, were tested for FHV-1 antigen using an immunofluorescent technique on air-dried conjunctival swabs. They were first treated with topical chlortetracycline with efficacy against *Chlamydophila* species and then, in cases positive for FHV-1, with topical aciclovir. The time to recovery was determined and illustrated using a Kaplan-Meier plot. Three cats were infected with *Chlamydophila* species and showed a median time to recovery of 14 days (95 per cent confidence interval [ci] 10 to 18 days), while 30 cats infected with FHV-1 showed a median time to recovery of 12 days (95 per cent ci 10 to 14 days). The drug dose at which 50 per cent plaque reduction (ED₅₀) occurred in a standard plaque reduction assay was determined in an in vitro study. This showed a mean (sd) ED₅₀ of aciclovir of 25 (3.5) mg/ml compared with 0.4 (0.05) mg/ml for trifluorothymidine, a drug known to be efficacious against FHV-1. The study shows that even though aciclovir is generally considered to lack efficacy against ocular FHV-1 infection, when used frequently it can have a beneficial effect in FHV-1 conjunctivitis and keratitis.

THE treatment of feline herpesvirus type 1 (FHV-1) keratitis and keratoconjunctivitis is complicated in the UK by the lack of efficacious licensed topical antiviral medications. Idoxuridine and vidarabine are widely used in the USA but are unavailable in the UK. The only drug currently available in the UK with proven efficacy against FHV-1 keratitis is trifluorothymidine, but it is not available through standard pharmaceutical wholesalers and, like other antiviral agents, is not licensed for use in cats. Aciclovir, a thymidine nucleoside analogue, is a widely available antiviral agent used for the treatment of herpes simplex virus infections in human beings, including ocular herpes, but has been discounted as a treatment for FHV-1 keratitis following one report of apparent inactivity of the drug in vitro (Nasise and others 1989). In the present study, aciclovir was shown to be effective in treating cats with FHV-1 keratitis. In vitro data investigating the efficacy of the drug, using a standard plaque resuction assay, are presented to support the in vivo findings.

A significant problem in determining the efficacy of treatments for ocular disease due to FHV-1 is that the severity of the condition often decreases during the natural course of the disease, potentially giving a false impression of improvement. A randomised, blinded trial comparing aciclovir and a placebo or aciclovir and trifluorothymidine, a drug with recognised efficacy against FHV-1, would have been the best method of determining the efficacy of aciclovir. But, in the first-opinion practice setting in which the trial was performed, this was not possible. Trifluorothymidine is only available in the UK from Moorfields Eye Hospital, a human ophthalmic hospital, on a named patient basis, and it has a short shelf-life. Immunofluorescent FHV-1 diagnosis was not available immediately and thus, given that the cats may have been infected with *Chlamydophila* species, a drug efficacious against that agent was employed initially. Thus, the cats were first treated with topical chlortetracycline until a diagnosis of FHV-1 infection was confirmed by the immunocytochemical detection of FHV-1 antigen or the ocular lesions resolved, suggesting a *Chlamydophila* species or bacterial infection. When a diagnosis of FHV-1 infection was made, the treatment

was changed to topical aciclovir, and the time for the clinical signs to resolve was measured as an indication of the efficacy of the drug.

MATERIALS AND METHODS

Thirty-three adult cats with ocular lesions that might have been due to FHV-1 or *Chlamydophila* species infections were included in the study. Their conditions were diagnosed by immunofluorescent antibody detection of FHV-1 antigen (Stiles and others 1997) and *Chlamydophila* antigen (Woodland and others 1978) on air-dried slides of conjunctival scrapes. For three weeks, from when they were first examined until the diagnoses were confirmed, the cats were treated with topical chlortetracycline ointment (Aureomycin; Cyanimid) three times a day. Thirty of the cats were diagnosed with FHV-1 and their treatment was changed to 0.5 per cent aciclovir ophthalmic ointment (Zovirax ophthalmic; Glaxo-SmithKline) five times a day. The interval from the start of this treatment to the resolution of the ocular signs was recorded on a Kaplan-Meier plot. Table 1 shows the age, sex, clinical signs, diagnosis and the time to the resolution of the clinical signs for each cat. A clinical score was obtained by summing the number of clinical signs, and the clinical signs were considered to have resolved when this score reached 0 or 1.

An investigation of the efficacy of aciclovir in vitro was also carried out. Feline embryo-derived cells were cultured to confluence in 24-well culture plates in Eagle's minimum essential medium (EMEM) + HEPES supplemented with 10 per cent fetal calf serum (FCS) and antibiotic and antifungal agents. To each well, an overlay of EMEM, supplemented with 1 per cent FCS, antibiotic and antifungal agents, and carboxymethylcellulose as a thickening agent, was added, together with a range of concentrations of aciclovir. The plates were seeded with a laboratory strain of FHV-1 (B927), incubated for 30 hours at 37°C, and then fixed and stained with a crystal violet/ethanol mix; the cytopathic effects were observed by light microscopy to identify plaques of dead cells. The efficacy

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TABLE 1: Age, sex and clinical signs in the 33 cats and the times taken by the lesions to resolve

Cat	Age (years)	Sex	Severity of conjunctivitis	Dendritic ulcer	Geographic ulcer	Vascular keratitis	Corneal sequestrum	FHV-1 confirmed	Time to resolution	
									Chlortetracycline (days)	Aciclovir (days)
1	6	Fn	+					+	NR	4
2	4	Fn	+					+	NR	6
3	7	Fn	++					+	NR	7
4	6	Fn	++					+	NR	7
5	4	Fn	++					+	NR	7
6	8	Fn	++					+	NR	8
7	3	Fe	++					+	NR	9
8	5	Mn	++					+	NR	11
9	2	Mn	++					+	NR	12
10	7	Fn	++					+	NR	17
11	5	Mn	++					+	NR	18
12	6	Mn	++					+	NR	18
13	8	Fn	++					+	NR	21*
14	4	Fe	+++					+	NR	14
15	5	Me	+++					+	NR	15
16	9	Mn	+++					+	NR	21
17	11	Fn	+++					+	NR	21
18	4	Fn	+++					+	NR	21
19	5	Mn	+++					+	NR	21
20	7	Fn	+	+				+	NR	7
21	6	Mn	+	+				+	NR	25*
22	3	Fn	+		+			+	NR	12
23	4	Fn	+		+			+	NR	13
24	7	Fn	+		+			+	NR	14
25	9	Mn	++		+			+	NR	16
26	4	Fn	++			+		+	NR	16
27	5	Fn	++			+		+	NR	17
28	7	Mn	+++			+		+	NR	22*
29	6	Mn	+				+	+	NR	14
30	3	Mn	++				+	+	NR	22*
31	7	Fn	++						9	NA
32	2	Fn	++						13	NA
33	8	Mn	++						24*	NA

* Poor compliance by the cat's owner

FHV-1 Feline herpesvirus type 1, NR No resolution, NA Not applicable, Fn Female neutered, Fe Female entire, Mn Male neutered, Me male entire

of the drug was determined by measuring the reduction in the number of viral plaques observed at the different concentrations of aciclovir, and the concentration at which the plaque reduction was 50 per cent (ED₅₀) was determined. The same standard plaque reduction assay was used to determine the ED₅₀ for trifluorothymidine, to enable comparison of the ED₅₀s for the two drugs.

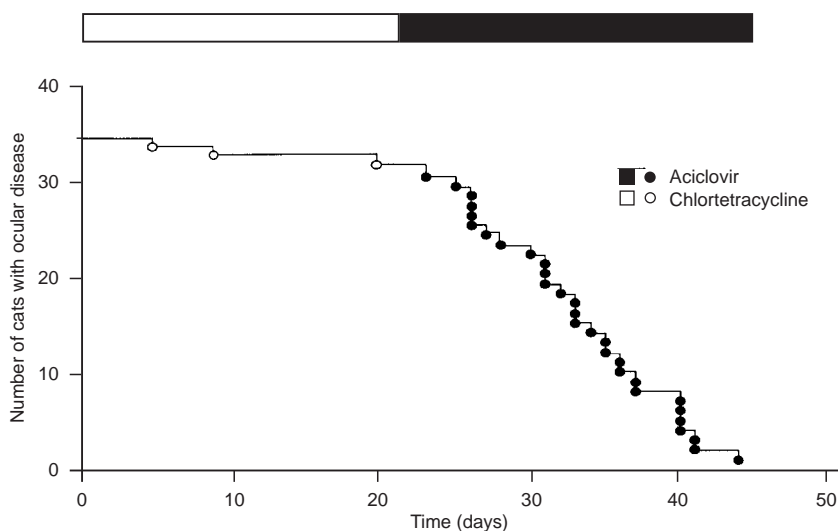


FIG 1: Kaplan-Meier plot of the number of cats treated for 21 days with chlortetracycline followed by a period of 21 days with aciclovir that continued to show clinical signs of ocular disease

RESULTS

Thirty of the cats tested positive for FHV-1 and three for *Chlamydomphila* species (Table 1). The clinical signs included conjunctivitis, dendritic corneal ulceration, superficial local corneal ulceration or vascular keratitis. The three *Chlamydomphila*-infected cats had conjunctivitis with varying degrees of chemosis. Of the 30 cats infected with FHV-1, 19 were affected solely by chronic conjunctivitis, two had dendritic ulceration, four had local ulceration and three had superficial keratitis; the other two cats were affected by a corneal sequestrum, and although the associated conjunctivitis resolved, the corneal lesion was removed by a superficial keratectomy. None of the cats infected with FHV-1 improved significantly during between two and three weeks of treatment with chlortetracycline, but their clinical signs either improved markedly or resolved completely after treatment with aciclovir for different periods.

In the three cats infected with *Chlamydomphila* species, the clinical signs resolved after between 10 days and three weeks of treatment with chlortetracycline. Fig 1 shows a Kaplan-Meier plot of the time to the resolution of the clinical signs during the treatments with chlortetracycline and aciclovir. The median time to resolution in the FHV-1 positive cats treated with aciclovir was 12 days (95 per cent confidence interval 10 to 14 days). The median time to the resolution of the signs of *Chlamydomphila* infection in the cats treated with chlortetracycline was 14 days (95 per cent confidence interval 10 to 18 days). Interviews with their owners when the cats were re-examined revealed that four of them had applied aciclovir three or fewer times daily; these animals are denoted by an asterisk in Table 1. When the frequency of drug administration was increased to five times a day, the clinical signs resolved.

Fig 2 shows the percentage plaque reductions produced by different concentrations of aciclovir and trifluorothymidine; the mean (sd) ED₅₀ of aciclovir was 25 (3.5) mg/ml and that of trifluorothymidine was 0.4 (0.05) mg/ml.

DISCUSSION

Ocular infections with FHV-1 are common, induce a variety of clinical signs and have a chronic time course (Nasisse 1982). Diseases due to herpesviruses often wax and wane, making it difficult to compare the efficacy of potential drugs, because the clinical signs may resolve spontaneously – as is the case with ‘cold sores’ in human beings. This is probably less of a problem with ocular infections in cats; corneal epithelial erosions and ulcers rarely recover spontaneously, although conjunctivitis may do so. In the present study the cats were first treated for three weeks with topical chlortetracycline, chosen because it would be efficacious in treating cases in which *Chlamydomphila* species rather than FHV-1 was the aetiological agent (Sparkes and others 1999); it also served as a control agent against which to compare aciclovir for its efficacy.

This trial has three potential shortcomings. First, it was not randomised, but in a first-opinion clinical environment such a protocol would have been neither practical nor ethically justifiable. Each cat was treated with topical chlortetracycline three times a day until a diagnosis was possible by the immunohistochemical demonstration of FHV-1 antigen in an air-dried conjunctival scrape. At that point the cats infected with FHV-1 were transferred to topical treatment with aciclovir ophthalmic ointment given five times a day. The confirmation of a viral aetiology took between two and three weeks, and all the cats were treated for three weeks with topical chlortetracycline to determine whether it was effective in FHV-1-infected animals or whether the clinical signs might resolve naturally. In the three cats in which *Chlamydomphila* species was detected there was an improvement in the ocular surface pathology and the conjunctivitis resolved, but there was no significant change in the other animals. However, when they were treated with aciclovir ophthalmic ointment five times a day, the ocular surface pathology associated with FHV-1 showed substantial improvement and in most cases the ocular signs resolved. In two cats with a corneal sequestrum the conjunctivitis improved but the sequestra did not resolve; they were removed by keratectomy.

Secondly, the trial should have been blinded, but the fact that one individual (D. L. W.) was responsible for the treatments and for the assessment of the therapeutic results rendered such a design impossible. Nevertheless, the severity of the clinical signs in many cases, including local corneal epithelial erosion, dendritic ulceration or profound vascular keratitis, the lack of effect of chlortetracycline, and the substantial resolution of these signs on aciclovir treatment, made the results unequivocal.

Thirdly, the ideal method of diagnosis of FHV-1 would have been the nested PCR. Stiles and others (1997) compared PCR, viral culture and immunofluorescent antibody detection, and found PCR to be significantly more sensitive than the other techniques. Nevertheless, although the immunofluorescent antibody technique is less sensitive than PCR, its specificity is not in question; a positive detection of viral antigen leads directly to a diagnosis of FHV-1 in association with the lesion.

However, the results conflict with the findings of Nasisse and others (1989) that aciclovir is ineffective in vitro. The relative efficacies of the drugs tested in that study were trifluridine >> idoxuridine > vidarabine > bromovinyl-deoxyuridine >> aciclovir, and the ED₅₀ for aciclovir was between 66.7 and 109 µM compared with between 0.5 and 0.96 µM for trifluorothymidine. In the present study the in vitro ED₅₀ for aciclovir was 25 mg/ml (83 µM) compared with an ED₅₀ of 0.4 mg/ml (1.35 µM) for trifluorothymidine, suggesting that aciclovir

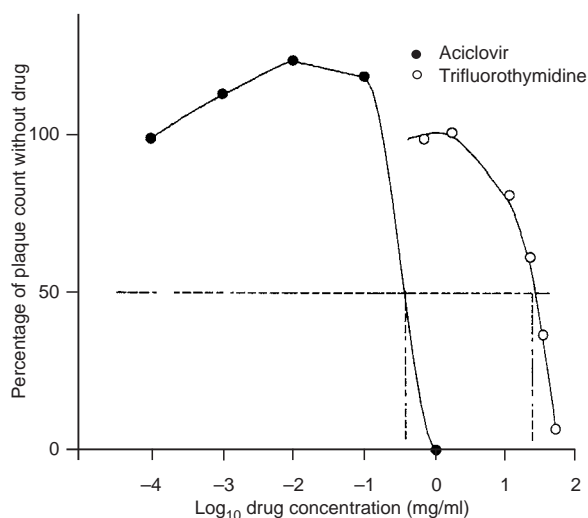


FIG 2: In vitro results showing the percentage reduction in viral plaque formation at different concentrations of trifluorothymidine and aciclovir

should be inactive in vivo, as was suggested by Nasisse and others (1989). Nevertheless, in a 0.3 per cent ointment formulation the effective concentration of aciclovir is 300 mg/ml, with a molarity of 12mM, given the molecular weight of aciclovir is 247 g/mol. Thus, even with a very high ED₅₀, the high drug concentration in the topical formulation should provide virotoxic potency on the ocular surface. The same cannot be said for the drug in the USA, where a topical formulation is not available and the oral formulation is sufficiently toxic to cats that to provide virotoxic tear levels causes systemic toxicity.

Nevertheless, the efficacy of topical aciclovir is still low and the treatment requires it to be administered five times a day to be fully effective. Other antiviral agents have a far lower ED₅₀ in in vitro studies and should provide an effective clinical treatment with a longer interval between doses and thus less inconvenience to the owner and stress to the cat. Other treatments suggested for ocular FHV-1 infection include alpha-interferon and oral lysine.

The results of this clinical trial, and the determination of the efficacy of aciclovir in vitro, show that topical aciclovir alone can be effective in resolving ocular surface lesions associated with FHV-1 infection.

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References

- NASISSE, M. P. (1982) Manifestations, diagnosis, and treatment of ocular herpesvirus infection in the cat. *Compendium on Continuing Education for the Practising Veterinarian* **4**, 962-970
- NASISSE, M. P., GUY, J. S., DAVIDSON, M. G., SUSSMAN, W. & DE CLERCQ, E. (1989) In vitro susceptibility of feline herpesvirus-1 to vidarabine, idoxuridine, trifluridine, acyclovir, or bromovinyldeoxyuridine. *American Journal of Veterinary Research* **50**, 158-160
- SPARKES, A. H., CANEY, S. M., STURGES, C. P. & GRUFFYDD-JONES, T. J. (1999) The clinical efficacy of topical and systemic therapy for the treatment of feline ocular chlamydia. *Journal of Feline Medicine and Surgery* **1**, 31-35
- STILES, J., MCDERMOTT, M., WILLIS, M., ROBERTS, W. & GREENE, C. (1997) Comparison of nested polymerase chain reaction, virus isolation, and fluorescent antibody testing for identifying feline herpesvirus in cats with conjunctivitis. *American Journal of Veterinary Research* **58**, 804-807
- WOODLAND, R. M., EL-SHEIKH, H., DAROUGAR, S. & SQUIRES, S. (1978) Sensitivity of immunoperoxidase and immunofluorescence staining for detecting chlamydia in conjunctival scrapings and in cell culture. *Journal of Clinical Pathology* **31**, 1073-1077