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NOTES

In Vitro Activities of Penciclovir and Acyclovir against Herpes Simplex Virus Types 1 and 2

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Penciclovir (PCV) and acyclovir are acyclic guanine analogs which inhibit herpes simplex virus (HSV) DNA polymerase. Their 50% infective doses were 0.5 to 0.8 μg/ml for clinical isolates of HSV-1 and 1.3 to 2.2 μg/ml for HSV-2. Furthermore, HSV-infected cultures receiving 2-h pulses of PCV had 2- to 50-fold less HSV than acyclovir-treated cultures, consistent with the prolonged intracellular half-life of PCV triphosphate.

Penciclovir [PCV; 9-(4-hydroxy-3-hydroxymethylbut-1-yl) guanine] is an acyclic guanine derivative with potent activity against herpes simplex viruses type 1 (HSV-1) and 2 (HSV-2), varicella-zoster virus, and Epstein-Barr virus (3). PCV is similar to acyclovir (ACV) in structure, metabolism, and antiviral spectrum. PCV, like ACV, is phosphorylated by a virus-specific thymidine kinase. PCV monophosphate is then phosphorylated by host enzymes to PCV triphosphate, which is a selective inhibitor of viral DNA synthesis. PCV triphosphate has an intracellular half-life of 10 h, which is 14 times longer than that of ACV triphosphate (5), resulting in antiviral activity persisting after the extracellular concentrations drop to low levels. In contrast, intracellular ACV triphosphate is rapidly metabolized to the acyclonucleoside which diffuses out of the cell. PCV triphosphate also achieves higher intracellular concentrations than ACV. The consequences of this difference were demonstrated by experiments showing that PCV reduced HSV yield with shorter periods of in vitro treatment than were required with ACV for the same effect (2). In addition, after removal of PCV from the medium, HSV replication remained inhibited for several days, whereas HSV replication resumed shortly after removing ACV. The persistence of PCV antiviral activity was confirmed by effectively treating HSV-infected mice with single daily doses of PCV (1).

We compared PCV with ACV for in vitro activity against additional clinical isolates of HSV-1 and HSV-2 and following intermittent or continuous exposure at concentrations close to their 50% in vitro inhibitory concentrations (ID_{50}s) for HSV.

Sensitivity of HSV clinical isolates to ACV and PCV. Twenty clinical isolates each of HSV-1 and HSV-2 from our clinical laboratory were grown and assayed by plaque titration in human embryonic lung fibroblast (HEL) tissue culture. ID_{50}s of the clinical isolates were determined by both plaque reduction assay (PRA) and enzyme-linked immunoassay (EIA) as previously described (4). The mean ± standard deviation ID_{50} values of ACV for HSV-1 isolates were 0.5 ± 0.4 μg/ml by PRA and 0.7 ± 0.5 μg/ml by EIA (Fig. 1). This was very similar to the sensitivity determined for PCV: 0.6 ± 0.4 μg/ml and 0.8 ± 0.3 μg/ml, by PRA and EIA, respectively. One HSV-1 isolate had intermediate sensitivity to ACV (ID_{50} of 2.2 μg/ml) and was sensitive to PCV (0.9 μg/ml). All others were sensitive to both drugs.

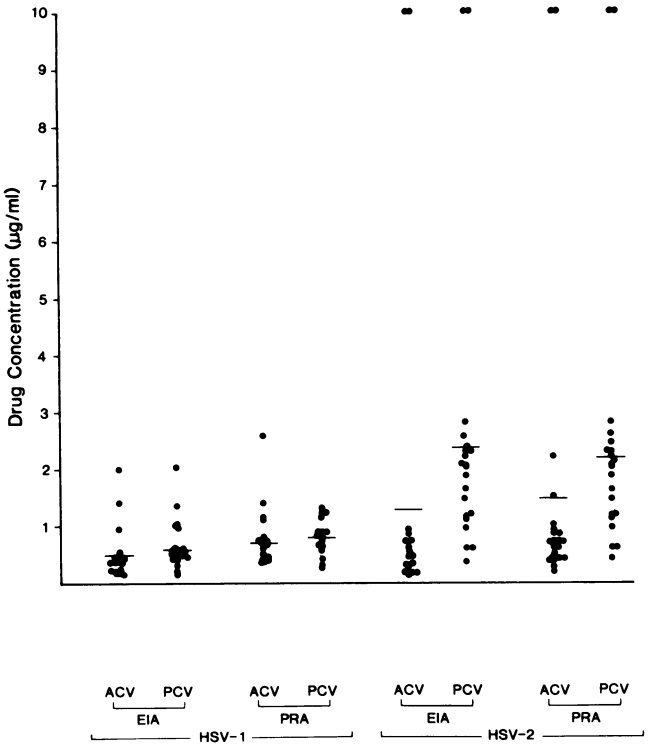

FIG. 1. Sensitivity of HSV clinical isolates to acyclovir (ACV) and penciclovir (PCV). Twenty clinical isolates each of HSV-1 and HSV-2 were tested by both EIA and PRA. The dots represent the ID_{50} of each HSV isolate. Horizontal bars represent means.

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TABLE 1. Titers of HSV in cell culture supernatants after intermittent in vitro treatment with ACV or PCV

Isolate	HSV type	Infectivity titer (10^5 PFU/ml) at 72 h (% of initial titer)[a]				
		Initial	ACV (1 μg)	PCV (1 μg)	ACV (2 μg)	PCV (2 μg)
11	2	0.5	1.2 (240)	0.2 (40)	8 (1,600)	0.1 (20)
20	2	22	5 (23)	1 (5)	2.2 (10)	0.4 (2)
45	2	370	370 (100)	210 (57)	520 (141)	160 (49)
15	1	10	26 (260)	14 (140)	2.8 (28)	0.8 (8)
28	1	87	270 (310)	1 (1)	40 (46)	7 (5)
41	1	170	370 (218)	210 (124)	36 (21)	4 (2)
55	1	66	90 (136)	18 (27)	1.8 (3)	1.4 (2)

[a] Data were derived from seven clinical isolates with intermediate sensitivity to ACV or PCV. The HSV isolates were grown in HEL cultures and exposed to 2-h pulses of ACV or PCV, at concentrations of 1 or 2 μg/ml, every 24 h. After 3 days, the viruses were harvested and titers determined by plaque titration in triplicate wells. The variance between replicates was less than 10%.

The drugs had comparable activity against HSV-2 isolates. ID_{50}s for ACV averaged 1.3 ± 2.8 μg/ml by PRA and 1.5 ± 2.7 μg/ml by EIA; for PCV the mean values were 2.4 ± 2.5 μg/ml and 2.2 ± 2 μg/ml. *t*-test analysis showed insignificant differences between the two drugs ($P = 0.4$). Three HSV-2 isolates had intermediate sensitivity to PCV, with ID_{50}s of 2.16 to 2.5 μg/ml, but were sensitive to ACV (ID_{50}s <1.5 μg/ml). Two isolates were resistant to both ACV and PCV, with ID_{50}s greater than 10 μg/ml.

Effect of intermittent in vitro treatment with ACV and PCV. To determine the effect of in vitro pulsing of HSV isolates with ACV and PCV at doses close to their ID_{50}s, seven isolates were selected with ID_{50}s between 1 and 1.5 μg/ml for HSV-1 and between 1.5 and 2 μg/ml for HSV-2. The isolates were inoculated onto HEL monolayers at a multiplicity of infection of 0.01. ACV or PCV, at final concentrations of 1 or 2 μg/ml, were added to each well for 2 h of every 24 h. Between pulses, the plates were washed three times and kept in maintenance medium. After 72 h, the titer of HSV in the infected cell suspension was determined (Table 1). PCV treatment at 2 μg/ml significantly decreased HSV titers below that of ACV-treated cultures by 74 ± 25% and below the initial titer by 88 ± 15% ($P = 0.04$ and 0.03, respectively); paired *t* test). At 1 μg/ml there was a trend toward lower titers in the PCV-treated isolates than the initial titers ($P = 0.09$). However, when HSV-2 isolates were analyzed separately, PCV at 1 μg/ml significantly decreased their titers ($P = 0.01$). In contrast, ACV did not alter the infectivity titers at either of the tested concentrations.

This finding is consistent with the 14-fold-greater intracellular half life of PCV triphosphate (5), which should inhibit virus long after the removal of extracellular PCV. Both ACV and PCV have serum half-lives of 2 h after intravenous administration of regular treatment doses. During much of the time that elapses between doses, the extracellular concentration of either drug is probably under the ID_{50} of many HSV isolates. These results suggest that PCV might be superior under these conditions and might permit administration of the drug at greater intervals.

Effect of continuous in vitro treatment with ACV or PCV on the titer of HSV isolates. The seven previously described isolates were cultured in medium containing 2 μg/ml ACV or PCV and maintained for 3 days without any medium change. Three isolates (one HSV-1 and two HSV-2) failed to grow in the presence of the antiviral agents. The viral infectivity in the remaining four cultures was measured by plaque titration (Table 2). The residual titer following PCV treatment (4.8 ± 0.3%) was less than that observed with ACV treatment (65.8 ± 74.2%; $P = 0.04$ by paired *t* test). These results could not be attributed to the intracellular persistence of PCV and indicate that PCV might have additional antiviral effects.

TABLE 2. Titer of HSV isolates after continuous in vitro treatment with ACV or PCV for 72 h

Isolate	Infectivity titer (10^5 PFU/ml) at 72 h (% of initial)[a]		
	Initial	ACV	PCV
20	22	38 (172.7)	0.3 (1.4)
28	87	27 (31)	15 (17.2)
41	170	7.9 (4.7)	0.2 (0.1)
55	66	36 (54.6)	0.4 (0.6)

[a] The seven HSV clinical isolates were grown for 3 days in HEL tissue culture in the presence of 2 μg/ml of ACV or PCV. Three isolates failed to grow in the presence of the antiviral agents. Virus titers were determined by plaque titration.

Effect of in vitro treatment with ACV or PCV on the sensitivity of HSV isolates. Intermittent in vitro treatment of HSV with either ACV or PCV did not select for resistance (ID_{50}s >3.5 μg/ml), but continuous treatment selected one resistant strain (isolate 28). Cross-resistance (ID_{50}s greater than 10 μg/ml) developed after both ACV and PCV treatment, which is in accordance with previous studies.

In conclusion, these in vitro studies with HSV clinical isolates suggested that PCV might be more effective than ACV in decreasing virus yield. It remains to be determined whether PCV may have clinical advantages over ACV.

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