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In Vitro Activity of Penciclovir against Clinical Isolates of Acyclovir-Resistant and Foscarnet-Resistant Herpes Simplex Virus

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We tested 23 clinical isolates of acyclovir-susceptible, acyclovir-resistant, and foscarnet-resistant herpes simplex virus for susceptibility to penciclovir. Isolates showed cross-resistance to penciclovir and acyclovir, but penciclovir retained a relative activity against foscarnet-resistant isolates. Its clinical utility for the treatment of resistant herpes simplex virus infections remains to be studied.

Penciclovir [9-(4-hydroxy-3-hydroxymethylbut-1-yl)guanine] is a nucleoside analog with in vitro activity against herpes simplex virus type 1 (HSV-1), HSV-2, and varicella-zoster virus (2, 4, 9). Despite a less powerful inhibition of the viral DNA polymerase by penciclovir triphosphate than by acyclovir triphosphate, the triphosphate ester of penciclovir within infected cells achieves higher and more prolonged intracellular concentrations (2, 8).

Ample clinical evidence has documented the occurrence of progressive mucocutaneous infection due to acyclovir-resistant HSV infection, particularly in immunosuppressed persons (6). Although the majority of such infections are due to deficient activity of the virus-specified thymidine kinase (TK), occasional clinical mutants with alteration in the DNA polymerase gene (5), as well as isolated patients with foscarnet-resistant HSV infection, have been described previously (1, 6a). TK-catalyzed triphosphorylation of penciclovir appears to be required for activation (2, 8), such that TK-deficient mutants of HSV or varicella-zoster virus would be expected to be cross resistant to acyclovir and penciclovir. However, one of seven clinical isolates of acyclovir-resistant varicella-zoster virus retained in vitro susceptibility to penciclovir in a recent study; this isolate was shown to have a mutation in the TK nucleoside binding site (7). Conceivably, therefore, the nucleoside binding sites of penciclovir and acyclovir may not be identical.

Comparative susceptibilities to penciclovir and acyclovir of resistant clinical mutants of HSV have not been reported to date. Therefore, we tested 23 clinical isolates of HSV which had been referred to the Herpes Virus Research Laboratory at San Francisco General Hospital for antiviral susceptibility testing, encompassing a spectrum of isolates highly susceptible to acyclovir (concentration of drug required to inhibit plaque formation by 50% [ID_{50}] < 1 μg/ml), isolates highly resistant to acyclovir (ID_{50} > 30 μg/ml), isolates of borderline susceptibility to acyclovir (ID_{50} = 1 to 3 μg/ml), and isolates resistant to foscarnet (ID_{50} ≥ 100 μg/ml), using the plaque reduction assay. Isolates resistant to acyclovir were susceptible to foscarnet (data not shown) and therefore presumed to contain a mutation within the TK gene. In contrast, foscarnet-resistant mutants are putative DNA polymerase mutants.

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Susceptibility was evaluated by seeding confluent monolayers of MRC-5 cells with 30 to 50 PFU of virus and then adding serial half-log concentrations of penciclovir, acyclovir, foscarnet, and/or ganciclovir. The ID_{50}s and ID_{90}s were calculated graphically. Acyclovir-susceptible (VL3-S), acyclovir-resistant (VL5-R), foscarnet-susceptible (KOS), and foscarnet-resistant (PAA^r5) laboratory control strains were also tested. Virus typing was performed by using fluorescein-labelled mouse monoclonal antibodies directed against HSV-1 and HSV-2 antigens (Syva Microtrak; Syva Company, Palo Alto, Calif.).

Seven different concentrations of penciclovir and acyclovir were tested; four different concentrations of foscarnet and ganciclovir were used. Each drug concentration was tested in triplicate. The coefficient of variation between wells for the 23 isolates tested for penciclovir susceptibility was 10% (10).

We found that ID_{50}s for penciclovir closely paralleled those for acyclovir (Spearman correlation coefficient = 0.79; $P < 0.001$), although values for penciclovir were typically higher than those for acyclovir against both acyclovir-susceptible and acyclovir-resistant isolates (Table 1). For acyclovir-susceptible isolates (ID_{50}, 0.12 to 0.9 μg/ml), the ID_{50} for penciclovir ranged from 0.21 to 2.3 μg/ml. For two isolates with borderline susceptibility to acyclovir (ID_{50}s, 2.1 and 2.2 μg/ml), the ID_{50}s for penciclovir were 3.1 and 3.7 μg/ml, respectively (Table 1). Isolates which were highly resistant to acyclovir (ID_{50}, 85 to 160 μg/ml) were similarly highly resistant to penciclovir (ID_{50}, 101 to 130 μg/ml).

Eight clinical isolates showed in vitro resistance to foscarnet (ID_{50}, 115 to 210 μg/ml) (Table 2). Of interest is that these isolates showed absolute or borderline susceptibility to acyclovir (ID_{50}, 0.37 to 3.0 μg/ml) as well as susceptibility to ganciclovir (ID_{50}, 0.12 to 1.1 μg/ml). Susceptibility to penciclovir was somewhat less pronounced (ID_{50}, 1.1 to 4.2 μg/ml).

Although previous studies have demonstrated a greater potency of both acyclovir and penciclovir against HSV-1 than against HSV-2 (ID_{50}s for acyclovir, 0.3 μg/ml for HSV-1 and 4.8 μg/ml for HSV-2 [3]; ID_{50}s for penciclovir, .5 to 0.8 μg/ml for HSV-1 and 1.3 to 2.2 μg/ml for HSV-2 [9]),

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TABLE 1. Comparative in vitro activities of penciclovir and acyclovir against clinical isolates of HSV[a]

Isolate or strain type and no.	Acyclovir		Penciclovir		HSV type
	ID_{50}	ID_{90}	ID_{50}	ID_{90}	
Acyclovir-susceptible isolates					
890520	0.12	0.17	0.21	0.54	1
910160	0.18	0.92	0.32	0.72	2
900400	0.19	0.74	0.57	3.9	2
900280	0.28	0.9	1.35	12.5	2
920027	0.33	0.9	0.9	3.6	2
900290	0.36	1.2	2.3	4.4	2
900020	0.5	3.7	2.0	10.0	2
890130	0.9	3.7	1.5	10.4	2
Isolates of borderline susceptibility to acyclovir					
920053	2.1	3.6	3.2	8.0	1
920059	2.2	10.0	3.7	15.0	2
Acyclovir-resistant isolates					
890490	85	230	101	430	2
900160	90	540	130	3,000	2
890600	100	420	101	370	2
890480	115	460	115	940	2
890654	160	540	120	480	2
Reference strains[b]					
VL3-S	0.35	0.8	0.35	1.2	1
VL5-R	12	58	90	280	1

[a] In micrograms per milliliter.

[b] Retesting of control strains yielded the following values (ID_{50} and ID_{90}, respectively, of each drug). For VL3-S: acyclovir, 0.45 and 1.0; penciclovir, 0.5 and 2.4. For VL5-R: acyclovir, 10 and 38; penciclovir, 79 and 190.

we were unable to evaluate differential susceptibility according to virus type because of the limited number of HSV-1 strains in this study (two clinical isolates and four laboratory control strains were HSV-1).

The prolonged intracellular half-life of penciclovir leading to persistence of antiviral effect may make possible a less frequent dosing interval than that required by acyclovir, with equivalent or superior efficacy (8, 9). Nevertheless, the cross-resistance to penciclovir and acyclovir of the five acyclovir-resistant clinical isolates tested, as well as the higher ID_{50}s found for penciclovir against the two isolates of borderline acyclovir susceptibility, does not encourage the expectation of efficacy for penciclovir against acyclovir-resistant HSV infection. Given the possibility of a different site of interaction for penciclovir and acyclovir on the TK enzyme (7), however, it is conceivable that occasional acyclovir-resistant isolates, such as those of the TK-altered phenotype, may retain susceptibility to penciclovir. Also, the relative susceptibilfty to both penciclovir and acyclovir of the eight foscarnet-resistant clinical isolates tested suggests that the binding site of penciclovir on the virus DNA polymerase may differ for foscarnet and penciclovir. The clinical utility of penciclovir against resistant HSV infections, as well as the molecular basis for such resistance, remains to be further investigated.

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TABLE 2. In vitro activities of penciclovir, acyclovir, and ganciclovir against foscarnet-resistant clinical isolates of HSV[a]

Isolate no. or strain	Acyclovir		Penciclovir		Foscarnet		Ganciclovir		HSV type
	ID_{50}	ID_{90}	ID_{50}	ID_{90}	ID_{50}	ID_{90}	ID_{50}	ID_{90}	
Isolates									
920068	3.0	11	2.5	6.6	115	410	1.1	5.8	2
90395	0.37	1.7	4.2	900	140	1,700	0.12	0.5	2
920023	1.4	5.2	1.1	27	150	1,000	0.14	0.8	2
920020	1.5	7.0	2.1	11	151	320	0.24	0.88	2
890546	2.1	25	1.6	100	160	1,000	0.25	0.5	2
90157	0.94	7.5	4.2	11	190	640	0.16	0.56	2
910580	1.0	4.2	3.0	12.7	210	540	0.12	0.6	2
900180	0.62	2.6	3.5	9.6	210	3,000	0.16	0.54	2
Reference strains									
KOS			0.96	4.0	27	47			1
PAA^r5			2.9	7.4	230	2,770			1

[a] In micrograms per milliliter.

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REFERENCES

1. **Birch, C. J., G. Tachedjian, R. R. Doherty, K. Hayes, and I. D. Gust.** 1990. Altered sensitivity to antiviral drugs of herpes simplex virus isolates from a patient with the acquired immunodeficiency syndrome. J. Infect. Dis. **162**:731–734.
2. **Boyd, M. R., T. H. Bacon, D. Sutton, and M. Cole.** 1987. Antiherpes activity of 9-(4-hydroxy-3-hydroxymethylbut-1-yl)guanine (BRL 39123) in cell culture. Antimicrob. Agents Chemother. **31**:1238–1242.
3. **Dorsky, D. I., and C. S. Crumpacker.** 1987. Drugs five years later: acyclovir. Ann. Intern. Med. **107**:859–874.
4. **Earnshaw, D. L., T. H. Bacon, S. J. Darlison, K. Edmonds, R. M. Perkins, and R. A. Vere Hodge.** 1992. Mode of antiviral action of penciclovir in MRC-5 cells infected with herpes simplex virus type 1 (HSV-1), HSV-2, and varicella-zoster virus. Antimicrob. Agents Chemother. **36**:2747–2757.
5. **Sacks, S. L., R. J. Wanklin, D. E. Reece, K. A. Hicks, K. L. Tyler, and D. M. Coen.** 1989. Progressive esophagitis from acyclovir-resistant herpes simplex. Clinical roles for DNA polymerase mutants and viral heterogeneity? Ann. Intern. Med. **111**:893–899.
6. **Safrin, S.** 1992. Treatment of acyclovir-resistant herpes simplex virus infections in patients with AIDS. J. Acquired Immune Defic. Syndr. **5**(Suppl. 1):S29–S32.

6a. **Safrin, S.** Unpublished data.

7. **Tallerico, C. L., W. Phelps, and K. K. Biron.** 1993. Analysis of the thymidine kinase genes from acyclovir-resistant mutants of varicella-zoster virus isolates from patients with AIDS. J. Virol. **67**:1024–1033.
8. **Vere Hodge, R. A.** 1993. Famciclovir and penciclovir. The mode of action of famciclovir including its conversion to penciclovir. Antiviral Chem. Chemother. **4**:67–84.
9. **Weinberg, A., B. J. Bate, H. B. Masters, S. A. Schneider, J. C. Clark, C. G. Wren, J. A. Allaman, and M. J. Levin.** 1992. In vitro activities of penciclovir and acyclovir against herpes simplex virus types 1 and 2. Antimicrob. Agents Chemother. **36**:2037–2038.
10. **Zar, J. H.** 1984. Measures of dispersion and variability, p. 27–39. *In* J. H. Zar (ed.), Biostatistical analysis. Prentice-Hall, Inc., Englewood Cliffs, N.J.