Activity of penciclovir in antiviral assays against herpes simplex virus

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The effect of penciclovir and acyclovir on the replication of herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) strains was determined in MRC-5 cells infected with 0.01 pfu/cell and exposed to the drugs for 72 h to allow multiple cycles of replication. Penciclovir was significantly more active than acyclovir against three strains of HSV-1 and three strains of HSV-2 at 1 mg/L (P = 0.009), 3 mg/L (P < 0.001) and 10 mg/L (P = 0.001). Further comparisons between the compounds were made in MRC-5 cells infected with HSV-1 strain SC16 using four different antiviral assays namely, the 24 h virus yield reduction assay, plaque reduction assay, viral antigen inhibition assay, and a viral DNA inhibition assay, to determine the relative merits of each. Penciclovir and acyclovir shared similar activities in the plaque reduction assay (with 50% effective concentrations, EC50, being 0.8 and 0.6 mg/L, respectively) and in the viral antigen inhibition assay (EC50, 0.6 and 0.7 mg/L, respectively). The EC50 of penciclovir in the 24 h viral DNA inhibition assay was 0.01 mg/L compared with 0.06 mg/L of acyclovir. In the 24 h virus yield reduction assay in which MRC-5 cells were infected with 0.3 pfu/cell, penciclovir was more active than acyclovir with 99% effective concentrations of 0.6 mg/L and 1.1 mg/L, respectively. The activity of penciclovir in the 24 h virus yield reduction and antigen inhibition assays was inversely related to the multiplicity of infection, whereas this had considerably less effect on the inhibition of viral DNA synthesis. These results suggest that famciclovir, which is the oral form of penciclovir, will be at least as effective as acyclovir in treating infections caused by HSV-1 and HSV-2.

Introduction

Penciclovir (9-(4-hydroxy-3-hydroxymethylbut-1-yl)guanine; BRL 39123) is a potent and selective inhibitor of herpes simplex virus type 1 (HSV-1), herpes simplex virus type 2 (HSV-2) and varicella-zoster virus cell in culture (Boyd et al., 1987; Boyd, Safrin & Kern, 1993). Both penciclovir (Boyd, Bacon & Sutton, 1988) and the oral form, famciclovir (Sutton & Kern, 1993) are effective in animals infected with HSV-1 or HSV-2. Famciclovir is the 6-deoxy, diacetyl ester of penciclovir (Vere Hodge et al., 1989) and has been approved for the treatment of herpes zoster in immunocompetent patients and has recently gained approval for use in patients with acute genital herpes infections.

Penciclovir is phosphorylated selectively to the monophosphate by viral thymidine kinase within herpesvirus-infected cells. Further phosphorylation by cellular enzymes

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results in penciclovir-triphosphate, which selectively inhibits viral DNA polymerase (Earnshaw et al., 1992; Vere Hodge & Cheng, 1993). The mechanism of action of acyclovir and penciclovir are qualitatively similar (Darby, 1993) although some quantitative differences have been noted in the efficiency of phosphorylation (Vere Hodge & Perkins, 1989; Earnshaw et al., 1992), the affinity of the viral DNA polymerases for the triphosphate form (Earnshaw et al., 1992; Bacon et al., 1993) and in the stability of the triphosphate in infected cells (Vere Hodge & Perkins, 1989; Earnshaw et al., 1992; Standing-Cox et al., 1994).

The plaque reduction assay is one of the most widely used for evaluating the activity of compounds for activity against HSV in cell culture by their inhibition of the cytopathic effect (cpe). Alternative tests have also been described such as the virus yield reduction assay (Collins & Bauer, 1977), the viral antigen inhibition assay (Wahren et al., 1983; Rabalais, Levin & Berkowitz, 1987) and the viral DNA inhibition assay (Gadler, Larsson & Solver, 1984; Swierkosz et al., 1987). In the present study, a 72 h virus yield reduction assay in MRC-5 cells infected with 0.01 pfu/cell of four clinical isolates and two laboratory strains of HSV-1 and HSV-2 was employed to compare penciclovir and acyclovir. This assay provides a stringent test by allowing multiple cycles of replication to occur before measuring the production of infectious virus. Penciclovir and acyclovir were also compared using four contrasting antiviral assays measuring different events in the viral replication cycle in order to evaluate the relative merits of each assay. Finally, experiments were performed to examine the effect of varying the multiplicity of infection on the ability of penciclovir to inhibit a single strain of HSV-1 in these assays.

Materials and methods

Test compounds

Penciclovir and acyclovir were synthesised in the laboratories of SmithKline Beecham Pharmaceuticals, Epsom, Surrey, UK. Stock solutions of 10 g/L of each compound were prepared in dimethyl sulphoxide and were stored at -20°C. When required, each drug solution was further diluted in growth medium (GM) consisting of Eagle’s Minimal Essential Medium (EMEM) containing 10% fetal calf serum. For the cytotoxicity assay, the compounds were dissolved in GM at 1 g/L.

Cells and viruses

MRC-5 cells were used for all experiments and were grown in GM. For the antiviral assays, cell monolayers were prepared in 96-well plates unless otherwise stated. HSV-1 SC16 (Hill, Field & Blyth, 1975) was provided by Dr H. J. Field (University of Cambridge, UK) and HSV-2 MS was purchased from the American Type Culture Collection (Maryland, USA). Clinical isolates (HSV-1 119, HSV-1 220, HSV-2 1458 and HSV-2 3218) were provided by a clinic in Rotterdam, the Netherlands.

Cytotoxicity assay

Replicating MRC-5 cells in 96-well plates were exposed to 0.03–100 mg/L of drug in GM for 2 days. Cells were then pulsed for 5 h with [6-\(^{3}\)]H]-thymidine (Amersham
International plc, Little Chalfont, Bucks, UK) and processed as previously described (Vere Hodge & Perkins, 1989), except that $^3$H-thymidine incorporation was measured using the Wallac Betaplate System (E.G. & G., Milton Keynes, Bucks, UK).

**Virus yield reduction assay**

The effect of each drug on the production of infectious virus was assessed in yield reduction assays where cells were infected with virus either at 0.3 pfu/cell and then treated for 24 h, or at 0.01 pfu/cell and then exposed to drug for 72 h. After virus adsorption, cultures were exposed in triplicate to serial half-log dilutions of the test compounds prepared in assay medium (AM) comprising EMEM containing 5% newborn calf serum as previously described (Boyd et al., 1987). Supernatants were pooled as appropriate either 24 h or 72 h after infection and cell-free virus infectivity titres were determined in duplicate by plaque assay in Vero cell monolayers. The end-point for the 24 h assay was the effective concentration ($EC_{90}$) which reduced virus yield by 99% in comparison with that of control cultures. No attempt was made to define an end-point for the 72 h virus yield assays because the shapes of the dose-response curves for penciclovir and acyclovir were different.

**Plaque reduction assay**

MRC-5 cell monolayers prepared in 24-well plates were infected with each of the HSV strains and exposed to the test compounds as described previously (Boyd et al., 1987), except that three replicates were tested for each dilution unless stated otherwise.

**Viral antigen inhibition assay**

MRC-5 cells were infected with HSV-1 SC16 at 0.01 pfu/cell and exposed in triplicate to various dilutions of the test compounds in AM for 48 h at 37°C. After supernatants were removed, 50 μL of 1% Triton X-100 in phosphate-buffered saline (PBS) was added to each well, and the plates were incubated at 37°C for 1 h. The solubilised antigen extracts were transferred to Immulon 2 Microtitration plates (Dynatech Laboratories Ltd., Billinghamurst, Sussex, UK) and adsorbed overnight at 4°C. The plates were washed three times with PBS containing 0.05% Tween 20 (PT) and non-specific binding sites blocked by the addition of 100 μL 0.2% gelatin in PBS for 30 min at 37°C, to each well. Plates were washed three times with PT containing 1% bovine serum albumin (PTB). Next, serum from a healthy volunteer seropositive for HSV-1 was added at a 1/50 dilution in PTB and incubated for 1 h at 37°C. After washing with PTB, a 1/500 dilution in PTB of peroxidase-conjugated goat anti-human Ig G antibody (Sigma, Poole, Dorset, UK) was added and incubated for 2 h at 37°C. Plates were again washed in PTB after which substrate consisting of 0.4 mM 3,3',5,5'-tetramethylbenzidine in 0.1 M sodium acetate/citric acid buffer pH 6.0 with 0.005% hydrogen peroxide was added and allowed to react for 1 h at room temperature. The reaction was stopped with 2 M sulphuric acid and absorbances were read at 450 nm (Dynatech Mini Reader II; Dynatech Laboratories Ltd., Billinghamurst, Sussex, UK). Plates were blanked against antigen extracted from uninfected cell monolayers, and the $EC_{50}$, i.e. the concentration required to inhibit viral antigen synthesis by 50% relative to the virus control, was determined from the dose-response curve for each compound.
Viral DNA inhibition assay

Monolayers of MRC-5 cells were infected with HSV-1 SC16 at 5 pfu/cell and exposed in triplicate to the test compounds for 24 h at 37°C as described for the virus yield reduction assay. Cells were then solubilised by adding 100 μL 1% sodium dodecyl sulphate in water, to each well. The lysates were transferred onto Hybond C-Extra filters (Amersham International plc) using a manifold. Viral DNA was quantified by hybridisation using a DNA probe specific for HSV, pHSV106 (Vere Hodge & Perkins, 1989). Autoradiographs were scanned with a laser densitometer (UltraScan XL, Pharmacia Biotech, St Albans, Herts, UK). To evaluate the effect of multiplicity of infection on the activity of penciclovir, cells were infected as above and exposed to the drug for 24 h after infection. Cell monolayers were then lysed and viral DNA was quantified by DNA hybridisation using the Hybriwix Probe System TM (Diagnostic Hybrids Inc., Athens, Ohio, USA) according to the manufacturer's instructions. Reagents for lysing cells as well as the filters and the 125I-labelled HSV-specific DNA probe were supplied in the kit. The EC50 was defined as the concentration of compound required to inhibit HSV DNA synthesis by 50% relative to the virus control.

Data analysis

Curves for the plaque reduction, antigen inhibition and DNA inhibition assays were fitted to the mean dose response expressed as a percentage of the virus control at each dose level using the RS-1 procedure #Fit Logistic (BBN Software Products Corporation, Cambridge, MA, USA). For the 24 virus yield reduction assays, EC95s were read from graphs of the virus yield plotted against drug concentration. Two-way comparisons between penciclovir and acyclovir in virus yield and plaque reduction assays were made using the Student's paired t-test. A P value of <0.05 was considered to be statistically significant.

Figure 1. Inhibition of the replication of clinical isolates of HSV-1 and HSV-2 in MRC-5 cells by penciclovir and acyclovir. Cells were infected with HSV-1 strain 220 (a) or HSV-2 strain 3218 (b) at 0.01 pfu/cell and cell-free virus titres quantified 72 h after infection. Each point represents the infectivity titre of the pooled supernatant from triplicate cultures. •, Penciclovir; O, acyclovir. Both clinical isolates used were fully susceptible to these compounds in the plaque reduction assay with EC95s of 0.9 mg/L penciclovir and 0.4 mg/L acyclovir for HSV-1 strain 220, and 1.1 mg/L penciclovir and 0.9 mg/L acyclovir for HSV-2 strain 3218.
Results

Penciclovir and acyclovir exerted a minimal effect on the incorporation of $^3$H-thymidine by replicating MRC-5 cells with IC$_{50}$s, the concentration of compound required to inhibit incorporation by 50%, being > 100 mg/L.

_Plaque reduction assay_

All the HSV strains used for this work were tested in the MRC-5 plaque reduction assay to verify that they were fully susceptible to both compounds. Mean EC$_{50}$s for penciclovir and acyclovir were not statistically significantly different being 0.6 and 0.4 mg/L respectively against the three HSV-1 strains, and 0.9 and 0.6 mg/L respectively against the three HSV-2 strains.

_Virus yield reduction 72 h assay following infection at low multiplicity_

Increasing concentrations of penciclovir (0.3–10 mg/L) produced progressively greater inhibition of HSV replication following infection of MRC-5 cells with HSV-1 or HSV-2 at 0.01 pfu/cell (Figure 1). In contrast, dose-response curves for acyclovir were less steep and plateaued at concentrations between 1 and 10 mg/L. In this assay penciclovir was significantly more effective than acyclovir in reducing the production of infectious virus for all strains tested at 1 mg/L ($P = 0.009$), 3 mg/L ($P < 0.001$), and 10 mg/L ($P = 0.001$) (Table I). At the lowest concentration (0.3 mg/L), penciclovir and acyclovir shared similar activity against most strains.

_Comparisons of penciclovir and acyclovir in antiviral assays against HSV-1 SC16_

HSV-1 SC16 was employed in order to evaluate the relative activities of penciclovir and acyclovir in the different antiviral assays. In the plaque reduction assay, penciclovir was marginally less active than acyclovir (Table II). Similar EC$_{50}$s were observed for penciclovir and acyclovir in the viral antigen inhibition assay (Table II). In both the viral DNA inhibition assay and 24 h virus yield reduction assay, penciclovir was more active than acyclovir (Table II).

_Antiviral activity of penciclovir in cultures infected at different multiplicities_

In the 24 h virus yield reduction assay, when cells were infected with 60 or 30 pfu/cell, dose-response curves were shallow with a substantial amount of virus being released over the range of concentrations tested (Figure 2(a)). Conversely, after infection at lower multiplicity, penciclovir showed greater inhibition of virus replication as is illustrated by a steeper dose-response curve. Thus, in the virus yield reduction assay, the efficacy of penciclovir was inversely related to the multiplicity of infection (Table III). The effect of penciclovir on HSV-1 antigen expression was also influenced by the multiplicity of infection (Figure 2(b)) and, as with the virus yield reduction assay, the activity of penciclovir was reduced after infection at high multiplicity (Table III). In contrast, when the virus challenge varied from 0.03 to 30 pfu/cell, the multiplicity of infection had a much smaller effect on the inhibition of HSV-1 DNA synthesis by penciclovir with EC$_{50}$s ranging from 0.1 to 1.0 mg/L (Figure 2(c)) (Table III). This observation was confirmed.
Table I. Effect of penciclovir and acyclovir in the virus yield reduction assay in MRC-5 cells infected at 0.01 pfu/cell. Penciclovir (PCV) was significantly more active than acyclovir (ACV) against HSV-1 and HSV-2 strains at 1 mg/L ($P = 0.009$), 3 mg/L ($P < 0.001$) and also at 10 mg/L ($P = 0.001$)

Mean infectivity titre 72 h after infection (log$_{10}$ pfu/0.1 mL)

<table>
<thead>
<tr>
<th>Virus strain</th>
<th>0.3 mg/L</th>
<th>1.0 mg/L</th>
<th>3.0 mg/L</th>
<th>10.0 mg/L</th>
<th>Untreated virus control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PCV</td>
<td>ACV</td>
<td>PCV</td>
<td>ACV</td>
<td>PCV</td>
</tr>
<tr>
<td>HSV-1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>119</td>
<td>5.11</td>
<td>5.98</td>
<td>3.90</td>
<td>4.72</td>
<td>3.78</td>
</tr>
<tr>
<td>220</td>
<td>5.58</td>
<td>5.98</td>
<td>4.00</td>
<td>5.15</td>
<td>3.74</td>
</tr>
<tr>
<td>SC16</td>
<td>4.79</td>
<td>5.30</td>
<td>3.18</td>
<td>4.85</td>
<td>3.65</td>
</tr>
<tr>
<td>HSV-2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1458</td>
<td>4.52</td>
<td>5.90</td>
<td>4.04</td>
<td>5.49</td>
<td>3.85</td>
</tr>
<tr>
<td>3218</td>
<td>3.93</td>
<td>4.00</td>
<td>3.36</td>
<td>3.52</td>
<td>1.93</td>
</tr>
<tr>
<td>MS</td>
<td>2.61</td>
<td>2.53</td>
<td>1.70</td>
<td>2.26</td>
<td>1.00</td>
</tr>
</tbody>
</table>

in a parallel experiment where viral DNA levels were quantified using the $^{32}$P-labelled DNA probe (results not shown).

Discussion

Penciclovir and acyclovir are both highly selective antiviral agents as determined in a range of human cell lines of differing tissue origin (Boyd et al., 1993). In the present study, penciclovir was marginally more active than acyclovir in the 24 h yield reduction assay using MRC-5 cells infected with HSV-1 SC16 at 0.3 pfu/cell, consistent with earlier findings (Boyd et al., 1987). However, far greater differences between these compounds were observed in the 72 h yield reduction assay in which cells were infected with 0.01 pfu/cell with either HSV-1 or HSV-2. When comparisons were made at single concentrations for all six strains, penciclovir was significantly more active than acyclovir at 1, 3 and 10 mg/L confirming and extending work reported previously (Weinberg et al., 1992; Bacon & Schinazi, 1993).

Table II. Inhibition of HSV-1 SC16 in MRC-5 cells

<table>
<thead>
<tr>
<th>Assay</th>
<th>Moi$^a$ (pfu/cell)</th>
<th>Duration of assay (h)</th>
<th>End-point activity (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Virus yield reduction</td>
<td>$3 \times 10^{-1}$</td>
<td>24</td>
<td>EC$_{50}$ 0.6 penciclovir</td>
</tr>
<tr>
<td>Plaque reduction</td>
<td>$3 \times 10^{-4}$</td>
<td>72</td>
<td>EC$_{50}$ 0.8 penciclovir</td>
</tr>
<tr>
<td>Viral antigen inhibition</td>
<td>$1 \times 10^{-2}$</td>
<td>48</td>
<td>EC$_{50}$ 0.6 penciclovir</td>
</tr>
<tr>
<td>Viral DNA inhibition$^b$</td>
<td>$5 \times 10^{9}$</td>
<td>24</td>
<td>EC$_{50}$ 0.01 penciclovir</td>
</tr>
</tbody>
</table>

$^a$moi, multiplicity of infection.

$^b$Viral DNA levels were quantitated by DNA hybridisation using a $^{32}$P-labelled DNA probe.

Note: Six replicates were used in each experiment.
Inhibition of HSV in cell culture

In clinical studies with the oral form of penciclovir, famciclovir, administration of 250 mg gave a plasma $C_{\text{max}}$ for penciclovir of 1.6 mg/L (Pue et al., 1994). The $C_{\text{max}}$ for acyclovir after an oral dose of 200 mg was 0.3 mg/L (de Miranda & Blum, 1983). For comparison, the $C_{\text{max}}$ for acyclovir was 5.7 mg/L for volunteers receiving 1000 mg valaciclovir, the oral prodrug of acyclovir currently under clinical evaluation for the treatment of HSV infections (Weller et al., 1993). Assuming that it is valid to extrapolate from human pharmacokinetic studies to cell culture experiments, these data indicate that the marked differences observed between penciclovir and acyclovir in the 72 h virus yield reduction assay occur at physiological concentrations.

In the 24 h virus yield reduction assay, the cultures probably support only one full round of HSV replication since the duration of the complete HSV replication cycle is approximately 18 to 20 h (Roizman & Sears, 1993). In contrast, several rounds of
Table III. Effect of multiplicity of infection on the inhibition of HSV-1 SCI6 in MRC-5 cells by penciclovir in different antiviral assays. Each assay was performed in triplicate and incubated for 24 h. HSV DNA levels in the cultures were measured by the Hybriwix Probe System.

<table>
<thead>
<tr>
<th>Initial moi* (pfu/cell)</th>
<th>Activity of penciclovir (mg/L)</th>
<th>Activity of acyclovir (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>virus yield reduction (EC₅₀)</td>
<td>viral antigen inhibition (EC₅₀)</td>
</tr>
<tr>
<td>60</td>
<td>&gt;30</td>
<td>&gt;30</td>
</tr>
<tr>
<td>30</td>
<td>2.8</td>
<td>0.9</td>
</tr>
<tr>
<td>6</td>
<td>0.9</td>
<td>0.9</td>
</tr>
<tr>
<td>3</td>
<td>0.6</td>
<td>17</td>
</tr>
<tr>
<td>0.6</td>
<td>0.6</td>
<td>—</td>
</tr>
<tr>
<td>0.3</td>
<td>—</td>
<td>2</td>
</tr>
<tr>
<td>0.06</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>0.03</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

*moi, multiplicity of infection.

replication are possible when cultures are infected with 0.01 pfu/cell and incubated for 72 h. Multiple cycles of replication could amplify a small difference in the ability of the two compounds to inhibit HSV replication.

Both compounds were active against HSV-1 SCI6 in MRC 5 cell cultures in all four antiviral assays, each of which measured a different virological end-point, but differences in the relative activities of penciclovir and acyclovir were observed between these tests. Penciclovir was more active in the 24 h virus yield reduction assay and viral DNA inhibition assay, observations consistent with previous work (Boyd et al., 1987; Vere Hodge & Perkins, 1989), whereas in the plaque reduction assay, acyclovir was marginally more active than penciclovir against HSV-1 SCI6, as has previously been observed in a series of 17 clinical isolates of HSV-1 using the same assay (Boyd et al., 1987). Both compounds were almost equally active in the viral antigen inhibition assay against HSV-1 SCI6 as has been reported elsewhere for clinical isolates of HSV-1 (Weinberg et al., 1992). Having chosen to vary the assay type but to use a single cell line and virus strain, it is possible that other inter-assay variations could have been observed with different combinations of cells and viruses. For instance, the cell line can influence the antiviral activity of a compound (Harmenberg, Wahren & Öberg, 1980; De Clercq, 1982; Boyd et al., 1993).

The activity of penciclovir was markedly reduced in cultures infected at high multiplicity when either virus yield (multiplicity of infection ≥30 pfu/cell) or viral antigen expression (multiplicity of infection ≥3 pfu/cell) was measured (Table III). The antiviral activity of acyclovir is also influenced by the multiplicity of infection in certain assays (Harmenberg et al., 1980, 1985). Littler (1994) compared penciclovir and acyclovir in 24 h virus yield reduction assays with HSV-1 and HSV-2 and showed that acyclovir and penciclovir had similar activity against HSV-1 SCI6 irrespective of multiplicity (10 and 0.01 pfu/cell). Against HSV-2 strain 186, acyclovir was clearly the more active compound when cultures were infected at 10 pfu/cell whereas this difference was much reduced after infection at 0.01 pfu/cell. IC₅₀s (concentrations causing 2-fold reduction in the yield of infectious virus) were calculated for these experiments (Littler, 1994), whereas EC₅₀s (concentrations causing 100-fold reduction in the yield of
infectious virus) were determined in the present report (Table II). Measurement of the
EC_{99} is therefore a more demanding test of antiviral efficacy than the EC_{50}. Moreover,
the EC_{99} is an appropriate end-point to use in the virus yield reduction assay because
virus replication can be inhibited by several orders of magnitude in this assay.

Changes in the initial multiplicity of infection had much less effect on the inhibition
of HSV-1 DNA synthesis by penciclovir, as has been reported elsewhere for acyclovir
(Swierkosz et al., 1987). This may be because the inhibition of viral DNA synthesis by
the triphosphates is the fundamental mechanism underlying the antiviral action of these
compounds (Furman et al., 1979; Earnshaw et al., 1992).

Consequently, an assay of the inhibition of viral DNA synthesis would be a good
choice for a routine susceptibility test, whereas initial multiplicity of infection would
need to be tightly controlled in the viral antigen inhibition or virus yield assays. Because
of the requirement to count discrete plaques in the plaque reduction assay the
multiplicity of infection must invariably be very low and the virus must be titrated
accurately beforehand. Moreover, whereas the DNA inhibition assay is objective, the
enumeration of plaques in the plaque reduction assay can be subjective. Nonetheless,
the plaque reduction assay is widely used to evaluate the effect of antiviral agents
against HSV.

Both the antigen inhibition and DNA inhibition assays are quick to perform and have
the potential for automation. The virus yield reduction assay is more time-consuming
to perform that the other tests evaluated but it provides important information on the
ability of a compound to inhibit infectious virus production. Of the four assays, it may
have the most clinical relevance in view of the importance of virus shedding in patients
with genital herpes. Moreover, the 72 h assay may be a better model for infection in
a patient than the 24 h assay because multiple rounds of replication can occur. The good
activity of penciclovir in cell culture, particularly in the 72 h virus yield reduction assay
in which multiple rounds of replication occur, suggests that famciclovir, the oral form
of penciclovir, will be at least as effective as acyclovir in treating both HSV-1 or HSV-2
infections.

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References

Inhibition of varicella-zoster virus by penciclovir—

Antiviral Research 20,
Suppl. 1, 109.

against herpes simplex virus and varicella-zoster virus in cell culture highlighting contrasts
with acyclovir. Antiviral Chemistry and Chemotherapy 4, Suppl. 1, 25-36.

hydroxymethylbut-1-yl)guanine (BRL 39123) in animals. Antimicrobial Agents and
Chemotherapy 32, 358-63.

9-(4-hydroxy-3-hydroxymethylbut-1-yl)guanine (BRL 39123) in cell culture. Antimicrobial


[9(4-hydroxy-3-hydroxymethylbut-1-yl)guanine: penciclovir]. Antimicrobial Agents and Chemotherapy 33, 1765–73.


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