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Antimicrobial Agents and Chemotherapy

2-[4,5-Difluoro-2-(2-Fluorobenzoylamino)-Benzoic Acid, an Antiviral Compound with Activity against Acyclovir-Resistant Isolates of Herpes Simplex Virus Types 1 and 2

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Herpes simplex viruses 1 and 2 (HSV-1 and HSV-2) are responsible for lifelong latent infections in humans, with periods of viral reactivation associated with recurring ulcerations in the orofacial and genital tracts. In immunosuppressed patients and neonates, HSV infections are associated with severe morbidity and, in some cases, even mortality. Today, acyclovir is the standard therapy for the management of HSV infections. However, the need for novel antiviral agents is apparent, since HSV isolates resistant to acyclovir therapy are frequently isolated in immunosuppressed patients. In this study, we assessed the anti-HSV activity of the antiadenoviral compounds 2-[2-(2-benzoylamino)-benzoylamino]benzoic acid (benzavir-1) and 2-[4,5-difluoro-2-(2-fluorobenzoylamino)-benzoylamino]benzoic acid (benzavir-2) on HSV-1 and HSV-2. Both compounds were active against both viruses. Importantly, benzavir-2 had potency similar to that of acyclovir against both HSV types, and it was active against clinical acyclovir-resistant HSV isolates.
commercially available as an oral agent (4, 9, 56). Famciclovir, the ester prodrug of penciclovir, increases the absolute bioavailability of penciclovir when administered orally (59).

Given that acyclovir and its derivatives are valuable antiviral agents with low toxicities, emerging drug-resistant HSV isolates are a major threat worldwide. The development of acyclovir resistance is much more common in immunosuppressed patients than in immunocompetent individuals. This is mainly due to long-term prophylactic acyclovir therapy in combination with an impaired host response that enables less virulent viruses to continue to replicate. The rate of isolation of acyclovir-resistant HSV in immunocompetent patients is reported to be between 0.1% and 0.6% (3, 8, 15), whereas drug-resistant HSV is found in 4% to 6% of immunosuppressed patients (13, 15, 19). However, an even higher frequency (36%) has been reported (34). The vast majority of drug-resistant isolates have mutations in the thymidine kinase (TK) gene (23), but alterations in the DNA polymerase gene have also been observed (16, 36). All TK-negative HSV isolates show cross-resistance between acyclovir and penciclovir, although some isolates with an altered TK gene are susceptible to penciclovir (9). When acyclovir or penciclovir therapy fails, the second line of defense is most often foscarnet followed by cidofovir therapy. Foscarnet is a phosphonic acid derivative that selectively binds and inhibits the pyrophosphate site on the herpesvirus DNA polymerase at concentrations that do not affect the cellular DNA polymerases. Foscarnet has been used successfully in managing acyclovir-resistant HSV infections (12, 41). However, emerging foscarnet-resistant isolates have been reported (46, 47). Cidofovir, a monophosphate form of an acyclic nucleoside analog that is TK independent, has been used in acyclovir- and foscarnet-resistant HSV infections (7, 13) but is known to be nephrotoxic and can be used only in patients with normal renal function.

Thus, there is a great need for the development of new antiviral drugs with novel targets. We have previously addressed this need by developing a cell-based viral replication assay and identifying several compounds that are active against human adenovirus (HAdV) (2). Similar to HSV infections, HAdV infections in immunocompetent individuals are mild and self limited, whereas in immunosuppressed patients they can cause life-threatening diseases (32, 51). HAdV infections have been reported in 5 to 21% of allogeneic bone marrow transplant recipients (22, 52). In addition, pediatric transplantation patients are at higher risk of developing disseminated HAdV infections, which are associated with high mortality rates (19 to 82%) (28). One of the compounds with promising results for screening of antiviral activity, 2-[4,5-difluoro-2-(2-fluorobenzoylamino)-benzoylamino]-benzoic acid (benzavir-2), proved to be a potent anti-HAdV compound with low toxicity for cells (2). Furthermore, the potency of this compound against HAdV was improved by iterative design, synthesis, and biological evaluation to furnish structure-activity relationships to the potent compound 2-[4,5-difluoro-2-(2-fluorobenzoylamino)-benzoylamino]-benzoic acid (benzavir-2) (2).

We addressed the activity of benzavir-1 and benzavir-2, with previously reported anti-HAdV activity, on HSV-1 and HSV-2. In addition, we assessed the activities on acyclovir-resistant isolates of both HSV-1 and HSV-2.

MATERIALS AND METHODS

HSV isolates and cells. The HSV isolates used in this study were from clinical samples collected from patients in Sweden (Table 1). The two acyclovir-sensitive isolates (of HSV-1 and HSV-2) were derived from separate patients and had been passaged 12 to 13 times in African green monkey kidney (GMK) cells at our clinical laboratory. The acyclovir-resistant isolates had been collected from five patients with recurrent herpetic lesions and had been sent to the virology laboratory of Sahlgrens University Hospital, Gothenburg, Sweden, due to suspected resistance to acyclovir. All five were found by plaque assay to be fully resistant to acyclovir. Four of these isolates were also tested by plaque assay for resistance to foscarnet and were found to be foscarnet sensitive. All HSV isolates were grown in GMK cells to produce viral stocks. These stocks were later quantified using quantitative PCR (qPCR) to determine the number of viral genomes per milliliter.

GMK cells were used throughout the study to assess HSV infection and the toxicities of the compounds. The cells were grown in Dulbecco’s modified Eagle medium (DMEM) (Sigma-Aldrich, St. Louis, MO) containing 0.75 mg/ml NaHCO₃, 20 mM HEPE (EuroClone, Milan, Italy), 1× PEST (100 IU/ml penicillin G, 100 μg/ml streptomycin sulfate; Gibco, Carlsbad, CA) and 5% fetal bovine serum (FBS) (Gibco) at 37°C.

FIG 1

The compounds assessed for anti-HSV activities in this study. Benzavir-1 was reported previously to have anti-HAdV activity (2) and has been optimized, using structure-activity relationship analysis, to give benzavir-2 (42).

TABLE 1

HSV isolates assessed in this study

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Type</th>
<th>Gender/age (yr)</th>
<th>Anatomical location of lesion</th>
<th>EC₅₀ of acyclovir</th>
<th>EC₅₀ of foscarnet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acyclovir-sensitive HSV</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HSV-1</td>
<td>1</td>
<td>M/22</td>
<td>Orolabial</td>
<td>1.6 μM⁺</td>
<td>ND⁻</td>
</tr>
<tr>
<td>HSV-2</td>
<td>2</td>
<td>F/24</td>
<td>Genital</td>
<td>1.6 μM⁺</td>
<td>ND⁻</td>
</tr>
<tr>
<td>Acyclovir-resistant HSV</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DE-5016</td>
<td>1</td>
<td>M/61</td>
<td>Corneal</td>
<td>&gt;80 μM</td>
<td>80 μM</td>
</tr>
<tr>
<td>DE-625</td>
<td>2</td>
<td>M/60</td>
<td>Knee pit</td>
<td>&gt;80 μM</td>
<td>ND⁻</td>
</tr>
<tr>
<td>DE-3657</td>
<td>2</td>
<td>F/52</td>
<td>Genital</td>
<td>&gt;80 μM</td>
<td>30 μM</td>
</tr>
<tr>
<td>DE-6820</td>
<td>2</td>
<td>F/83</td>
<td>Genital</td>
<td>80 μM</td>
<td>50 μM</td>
</tr>
<tr>
<td>DE-14574</td>
<td>2</td>
<td>F/82</td>
<td>Genital</td>
<td>&gt;80 μM</td>
<td>125 μM</td>
</tr>
</tbody>
</table>

*The acyclovir-resistant isolates were analyzed with a plaque assay at Sahlgrenska University Hospital, Gothenburg, Sweden. The reference values for acyclovir and foscarnet resistance were >4 μM and >160 μM, respectively.
⁺ EC₅₀ as presented in Fig. 3, analyzed by qPCR.
⁻ ND, not determined.
Compounds. Benzavir-1 and benzavir-2 were synthesized as described previously (42). Acyclovir and cidofovir were purchased from Sigma-Aldrich (Schnelldorf, Germany) as a powder. The compounds were dissolved in dimethyl sulfoxide (DMSO) in 20 mM stock solutions (benzavir-1, benzavir-2, and cidofovir) or 200 mM stock solution (acyclovir) and were stored at room temperature in the dark and in a dry atmosphere.

Quantitative real-time PCR for assessment of antiviral activity. On the day before infection, approximately 7.5 × 10^6 GMK cells were seeded in 24-well plates (Nunc) in DMEM with 5% FBS. On the day of infection, the cells in one well were counted in a Bürker chamber to establish the number of viral genomes (virions) per cell to be added. Amounts of the acyclovir-sensitive HSV-1 and HSV-2 viruses corresponding to 75 viral genomes/cell were added to the cells simultaneously with the addition of test compound in DMEM containing 2% FBS. For the acyclovir-resistant isolates, the numbers of viral genomes added per cell were normalized so that the replicated numbers of viral genomes were similar to the qPCR readouts of the acyclovir-sensitive HSV-1 and HSV-2 isolates (approximately 3,000 viral copies/cell). For the acyclovir-resistant isolates DE-625 and DE-6820, 20 copies per cell were added, for DE-3657, 25 copies per cell were added, for DE-14574, 50 copies per cell were added, and for DE-5016, 30 copies per cell were added to achieve a final number of 3,000 replicated viral copies/cell. The compounds were added simultaneously in concentrations ranging from 0.020 M to 20 M per well, and the plate was incubated at 37°C in 5% CO2 for 24 h. The final concentration of DMSO was less than 0.5% in all samples.

Twenty-four hours later, the medium was collected, and the cells were harvested (using 0.05% EDTA–phosphate-buffered saline [PBS] and 0.05% trypsin in 0.05% EDTA-PBS), and the total DNA in the medium and cells was extracted using the QIAamp DNA blood minikit (Qiagen, Solna, Sweden) according to the manufacturer’s instructions. The principle of qPCR has been described elsewhere (27), as has the design of primers and probes for the detection of HAdV have been described (1). The forward primer used for HAdV quantification. The design and analysis of primers and probes for the detection of HAdV was AdC (5'-AGG ACG CCT CGG AGT ACC TGA GCC GCA CCA G-3') and reverse primer (5'-CAT CAT TAT CCT CTT TAT CAT CAG CAC CA-3'). A standard curve was generated by serial dilution of known amounts of HAdV DNA or 10^6 genomes. The amplification of viral genomes per cell treated with 15 or 5 M acyclovir was determined by quantitative PCR (qPCR) using a variable slope using GraphPad Prism software version 5.0 (GraphPad Software, San Diego, CA).

RESULTS

Benzavir-1 and its analog benzavir-2 at 15 and 5 μM have potent antiviral activities against HSV-1 and HSV-2. The antiviral activities of 2-[2-(2-benzoylamino)-benzoylamino]benzoic acid (benzavir-1) and 2-[4,5-difluoro-2-(2-fluorobenzoylamino)benzoic acid (benzavir-2) were initially screened at 15 and 5 μM, and compared with the activities of acyclovir on acyclovir-sensitive isolates of both HSV-1 and HSV-2. The antiviral efficacy of the compounds was determined by quantitative PCR (qPCR) throughout the study. The percent inhibition of replication of viral genomes per cell treated with 15 or 5 μM acyclovir, benzavir-1, or benzavir-2, compared to infected but untreated GMK cells, is presented (Fig. 2). For HSV-1, a nearly complete inhibition (>93%) of the viral replication was observed for acyclovir at both 15 and 5 μM (Fig. 2A). However, for HSV-2, acyclovir appeared to be slightly less potent, with 80% inhibition at 5 μM (Fig. 2B). Both benzavir-1 and benzavir-2 had similar activities against HSV, regardless of type, and the overall results indicate that benzavir-2 is more potent than benzavir-1, which is in line with the activity against HAdV (42).

Acyclovir and benzavir-2 have similar EC_{50}s but different inhibitory profiles. Since acyclovir and both benzavir-1 and benzavir-2 almost completely abolished the replication of HSV at 15 μM and only minor differences were observed at 5 μM (Fig. 2), the efficacies were assessed by determining the half-maximum effective concentrations (EC_{50}s) on HSV-1 and HSV-2 (Fig. 3). The dose-response curves for acyclovir, cidofovir, benzavir-1, and benzavir-2 with HSV-1 and HSV-2 are shown in Fig. 3. The efficacies of acyclovir and benzavir-2 were the same irrespective of HSV type (acyclovir, EC_{50} = 1.6 μM for both HSV-1 and HSV-2; benzavir-2, EC_{50} = 1.5 μM for HSV-1 and EC_{50} = 1.6 μM for HSV-2). However, the efficacy of benzavir-1 was lower, and a
A slight difference was seen between HSV types (HSV-1, EC$_{50}$ = 3 μM; HSV-2, EC$_{50}$ = 4.3 μM). The efficacy of cidofovir was low against HSV-1 (EC$_{50}$ = 11 μM). Although the dose-response curves for benzavir-1 and benzavir-2 followed a sigmoidal shape and the curves for acyclovir were straighter, the EC$_{50}$s were similar for acyclovir and benzavir-2.

**Benzavir-1 and benzavir-2 have antiviral activity against acyclovir-resistant clinical isolates of HSV-1 and HSV-2.** In view of the facts that benzavir-1 and benzavir-2 had potent antiviral effects against both HSV-1 and HSV-2, and that benzavir-2 and acyclovir had similar EC$_{50}$s (Fig. 3), we investigated their activities against acyclovir-resistant clinical isolates (Table 2). The initial assessment was performed at concentrations of 15 and 5 μM. We assessed four HSV-2 isolates and one HSV-1 isolate, all of which were acyclovir resistant (Table 1). As expected, acyclovir had no or little effect on the replication of these isolates. A modest antiviral activity was seen against the HSV-1 isolate DE-5016 and HSV-2 isolates DE-14574 and DE-6820 (with approximately 20% inhibition at both 15 and 5 μM). On the other hand, benzavir-1 and benzavir-2 had potent activities against all isolates. However, the activity of benzavir-1 on DE-625 was low (43% inhibition at 15 μM and 13% at 5 μM). Benzavir-1 showed the highest overall activity against isolates DE-3657 and DE-6820 at 15 μM (97% inhibition for both). Benzavir-2 was more potent than benzavir-1.
Benzavir-2 showed an overall inhibition of >82% at 15 μM and >72% at 5 μM for all 5 isolates.

The efficacy of benzavir-2 against acyclovir-resistant isolates is in the low micromolar range. All the previous results indicated that benzavir-1 and benzavir-2 were potent inhibitors of HSV replication and that they were active against acyclovir-resistant isolates of HSV. In addition, our previous structure-activity relationship analysis of benzavir-1 had indicated that benzavir-2 was a more potent inhibitor of HAdV replication than benzavir-1 (42).

In the present study, on HSV replication, the results also indicated that benzavir-2 is a more potent antiviral agent than benzavir-1 (Fig. 2 and 3), and interestingly, it was also more effective against acyclovir-resistant isolates (Table 2). We therefore concentrated on benzavir-2 and established its efficacy on acyclovir-resistant isolates. As seen in Table 3 and Fig. 4, benzavir-2 showed high potency against all acyclovir-resistant isolates, with an average EC_{50} of ~1.3 μM for all isolates.

The ratio between the antiviral activity and the cellular toxicity of benzavir-2 is high. When the antiviral activity in cell-based systems is evaluated, a very important factor to be considered is the cellular toxicity. Compounds that interfere with essential cellular pathways or processes will eventually cause cell

![Dose-response curves](http://aac.asm.org/).
TABLE 2 The antiviral activities of benzavir-1 and benzavir-2 against acyclovir-resistant isolates of HSV-1 and HSV-2a

<table>
<thead>
<tr>
<th>Isolate-resistant HSV</th>
<th>Acyclovir inhibition (%) at:</th>
<th>Benazavir-1 inhibition (%) at:</th>
<th>Benazavir-2 inhibition (%) at:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15 μM</td>
<td>5 μM</td>
<td>15 μM</td>
</tr>
<tr>
<td>DE-5016</td>
<td>1</td>
<td>24 ± 10</td>
<td>20 ± 1</td>
</tr>
<tr>
<td>DE-625</td>
<td>2</td>
<td>0 ± 2</td>
<td>3 ± 4</td>
</tr>
<tr>
<td>DE-3657</td>
<td>2</td>
<td>6 ± 9</td>
<td>5 ± 7</td>
</tr>
<tr>
<td>DE-6820</td>
<td>2</td>
<td>11 ± 16</td>
<td>20 ± 11</td>
</tr>
<tr>
<td>DE-14574</td>
<td>2</td>
<td>28 ± 5</td>
<td>18 ± 7</td>
</tr>
</tbody>
</table>

a Based on three independent experiments, the percent inhibition values at 15 or 5 μM are given, along with standard deviations.

death. Such compounds will give false-positive results when inhibition of intracellular replication of viruses is analyzed. To ensure that the observed antiviral activities of benzavir-1 and benzavir-2 were not due to toxicity, XTT toxicity tests were performed (45). The XTT toxicity test quantifies the ability of mitochondrial enzymes to reduce 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide (XTT) to a colored formazan dye. The XTT toxicity test quantifies the ability of mitochondrial enzymes to reduce 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide (XTT) to a colored formazan dye. After 24 h of treatment with acyclovir, benzavir-1, and benzavir-2, the percentages of viable GMK cells were assessed (Table 4). We decided to analyze the cellular viability at 20 times (30 μM) and 40 times (60 μM) the EC50 concentration of benzavir-2 due to observed compound precipitation when the concentration reached 150 μM (unpublished data). Acyclovir did not have any negative effect on cell viability at these concentrations. However, benzavir-1 reduced the viability to 87% of DMSO controls at 30 μM and to 63% at 60 μM. Benzavir-2 showed low toxicity and reduced the viability to approximately 90% at 20 times the EC50 concentration and 83% at 40 times the EC50 concentration. This gives a favorable ratio between antiviral activity and toxicity for benzavir-2.

DISCUSSION

Despite the fact that acyclovir and its derivatives have been successful drugs in the management of HSV infections, HSV remains a major global health problem. It causes great morbidity in immunocompetent hosts and is associated with fatality in immunosuppressed patients, and it is a contributory factor in the spread of HIV (39, 62). Few other antiviral agents besides acyclovir are available for HSV, and isolates that are resistant to acyclovir therapy are frequently isolated from immunosuppressed patients (13, 34). Thus, there is an obvious need for new antiviral drugs against HSV infections.

In this study, we assessed the antiviral activity of two compounds (Fig. 1) with previously reported anti-HAdV activity against HSV, another DNA virus of medical importance. Using a whole-cell-based small-molecule screening assay, we have identified benzavir-1 as a novel anti-HAdV compound with low cellular toxicity (2). In the screening, benzavir-1 efficiently reduced the expression of green fluorescent protein (GFP) from a replication-competent GFP-expressing vector based on HAdV type 11. Moreover, benzavir-1 was equally effective against representatives of all HADV species, with a mean EC50 of 3.5 μM, and according to qPCR was approximately five times more efficient than cidofovir. Subsequently we applied iterative design, synthesis, and biological evaluation to generate structure-activity relationships and the improved compound benzavir-2 (42). Benzavir-2 was found to have an EC50 of 0.6 μM against HAdV type 5 and had a better cell viability profile than benzavir-1. In the present study, we found that benzavir-1 and benzavir-2 inhibited the replication of HSV to the same extent as they inhibited HAdV replication. The potential antiviral activity of benzavir-2 against other types of viruses will be assessed in planned studies. Benzavir-2 had an EC50 similar to that of acyclovir for both HSV-1 and HSV-2 and was approximately seven times more potent than cidofovir on HSV-1. However, the profiles of the dose-response curves differed between benzavir-2, acyclovir, and cidofovir (Fig. 3). Benzavir-1 and benzavir-2 inhibited HSV replication in a sigmoidal manner (with average slope parameters of −4.7 and −3.4, respectively) whereas the inhibitory profiles of acyclovir and cidofovir were more linear (slope parameters of −0.9 and −1.3, respectively). This observed difference in the dose-response curves might indicate that the mechanisms of action of benzavir-1 and benzavir-2 are different from those of acyclovir and cidofovir.

Interestingly, both benzavir-1 and benzavir-2 showed antiviral activities against clinical acyclovir-resistant isolates (Table 2) that were comparable to those against acyclovir-sensitive isolates (Fig. 2). Furthermore, the efficacy of benzavir-2 was the same for acyclovir-sensitive isolates (Fig. 3) and acyclovir-resistant isolates (Table 3 and Fig. 4). Acyclovir is a guanosine analog with a 2-hydroxyethylmethyl group that is selectively phosphorylated by the viral HSV thymidine kinase to its monophosphate form. This monophosphate form is further converted by cellular kinases to the triphosphate form, which is selectively incorporated into the virus DNA by the viral DNA polymerase. Due to the absence of a 3' end, the triphosphate form of acyclovir causes chain termination of viral DNA polymerization (18). The structures of benzavir-1 and benzavir-2 are fundamentally different from the structures of the nucleoside analog acyclovir, suggesting that the mechanism of action for benzavir-1 and benzavir-2 may not be the same as they inhibited HAdV replication. The potential antiviral activity of benzavir-2 against other types of viruses will be assessed in planned studies. Benzavir-2 had an EC50 similar to that of acyclovir for both HSV-1 and HSV-2 and was approximately seven times more potent than cidofovir on HSV-1. However, the profiles of the dose-response curves differed between benzavir-2, acyclovir, and cidofovir (Fig. 3). Benzavir-1 and benzavir-2 inhibited HSV replication in a sigmoidal manner (with average slope parameters of −4.7 and −3.4, respectively) whereas the inhibitory profiles of acyclovir and cidofovir were more linear (slope parameters of −0.9 and −1.3, respectively). This observed difference in the dose-response curves might indicate that the mechanisms of action of benzavir-1 and benzavir-2 are different from those of acyclovir and cidofovir.

TABLE 3 The efficacy of benzavir-2 against acyclovir-resistant HSV isolatesa

<table>
<thead>
<tr>
<th>Acyclovir-resistant HSV</th>
<th>Type</th>
<th>Benzavir-2 EC50</th>
</tr>
</thead>
<tbody>
<tr>
<td>DE-5016</td>
<td>1</td>
<td>1.1 μM</td>
</tr>
<tr>
<td>DE-625</td>
<td>2</td>
<td>1.2 μM</td>
</tr>
<tr>
<td>DE-3657</td>
<td>2</td>
<td>1.4 μM</td>
</tr>
<tr>
<td>DE-6820</td>
<td>2</td>
<td>1.4 μM</td>
</tr>
<tr>
<td>DE-14574</td>
<td>2</td>
<td>1.6 μM</td>
</tr>
</tbody>
</table>

a The EC50 values of benzavir-2 are based on three independent experiments with each concentration point in duplicate. Concentrations ranging from 20 μM to 0.156 μM, in 2-fold dilution steps (Fig. 4), were tested, and the EC50 values were calculated with nonlinear regression (log concentration of the test compound against response) with variable slopes.
ery. However, given the previously reported anti-HAdV activity of benzavir-2, the mechanism of action cannot, as with acyclovir, be specific for HSV infections. Accordingly, the target of benzavir-2 may well be shared or be homologous in HAdV- and HSV-infected cells. The potential development of resistance against benzavir-2, in both HAdV and HSV isolates, will be addressed in planned studies that may contribute to the elucidation of the target of benzavir-2. Due to the fact that both HAdV and HSV replication are also dependent on the host cell machinery, we cannot rule out a possible interference between benzavir-2 and cellular components. This could explain the observed effect on cell viability in the presence of high concentrations of benzavir-2 (Table 4). Ideally, an antiviral agent should be specific for virally encoded targets or, as for acyclovir, be activated by viral proteins. However, the specificity against the target is generally reduced with increasing drug concentrations. The nonnucleoside pyrophosphate analog foscarnet, which does not require virus-specific intracellular phosphorylation, indiscriminately inhibits cellular DNA polymerases at a 100-fold-greater concentration than viral polymerases (60). Foscarnet has been associated with serious side effects, most commonly nephrotoxicity, which affects 30% of patients (43). The favorable property of acyclovir, the requirement for an initial phosphorylation by the viral thymidine kinase to be activated, increases the specificity of acyclovir dramatically. A 3,000-fold-higher concentration of acyclovir is needed to inhibit the growth of the host cell compared to the inhibition of viral multiplication. However, in its triphosphate form, the selectivity between inhibition of the viral and cellular DNA polymerases is only 10 to 30 times (18). With this in mind, the low effect on the cellular viability of acyclovir (Table 3) can be explained by the absence of initial phosphorylation by the viral thymidine kinase that triggers further phosphorylations and results in the biologically active triphosphate form that terminates the DNA polymerization.

To conclude, both benzavir-1 and benzavir-2 are potent inhibitors of HSV replication, and they are active against acyclovir-sensitive and acyclovir-resistant isolates of both HSV-1 and HSV-2. Benzavir-2 is more potent than benzavir-1, with an efficacy similar to that of acyclovir, and this compound should be assessed further as a prelude to determining potential use in the possible management of acyclovir-resistant HSV infections.

ACKNOWLEDGMENTS

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REFERENCES


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**TABLE 4** The effect of acyclovir, benzavir-1, and benzavir-2 on cell viability

<table>
<thead>
<tr>
<th>Concn</th>
<th>Acyclovir (Viability % ± SD)</th>
<th>Benzavir-1 (Viability % ± SD)</th>
<th>Benzavir-2 (Viability % ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 µM</td>
<td>99.9 ± 2.5</td>
<td>87.3 ± 1.9</td>
<td>89.7 ± 2.3</td>
</tr>
<tr>
<td>60 µM</td>
<td>99.7 ± 5.7</td>
<td>62.6 ± 1.8</td>
<td>83.2 ± 2.8</td>
</tr>
</tbody>
</table>

*Cell viability was assessed in GMK cells using the XTT-based toxicity test at 20 and 40 times the EC_{50} of benzavir-2. The percent viability values given were normalized against DMSO controls with the same amount of DMSO as used for the compounds.*


38. Singh N, et al. 1988. Infections with cytomegalovirus and other her-


