Synergistic Effect of Ganciclovir and Foscarnet on Cytomegalovirus Replication In Vitro

JODY F. MANISCHEWITZ,* GERALD V. QUINNAN, JR., 1 H. CLIFFORD LANE,2 AND ALEC E. WITTEK1

Laboratory of Herpesvirus Research, Center for Biologies Evaluation and Research, Food and Drug Administration,1 and Laboratory of Clinical Immunology, National Institute of Allergy and Infectious Diseases,2 Bethesda, Maryland 20892

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Ganciclovir and foscarnet possess substantial activity against cytomegalovirus. Both exhibit dose-limiting toxicity, which reduces their clinical usefulness. We demonstrated synergistic inhibition of cytomegalovirus replication in vitro by ganciclovir and foscarnet. Reduced-dose combination therapy may provide a means to treat patients with cytomegalovirus infection while reducing drug toxicity.

Cytomegalovirus (CMV) infection is an important cause of morbidity and mortality in patients with acquired immunodeficiency syndrome and other immunodeficiency states, particularly after transplantation (6, 12, 13). Syndromes attributed to CMV include interstitial pneumonitis, hepatitis, thrombocytopenia, colitis, and chorioretinitis. Given the life-threatening nature of CMV infection in immunosuppressed patients, the development of more effective and safer antiviral drugs for the treatment of CMV disease would represent a major advance in therapeutics and could lead to increased survival and improved quality of life.

Treatment of CMV infections with vidarabine and acyclovir has not been successful, since inhibitory levels of drug are difficult to achieve (2, 19). The guanosine analog ganciclovir [9-(1,3-dihydroxy-2-propoxymethyl)guanine; DHPG] is a congener of acyclovir that has 10- to 100-fold more activity than acyclovir against CMV (9, 11, 14). Trials in patients with acquired immunodeficiency syndrome have shown that DHPG can suppress viral replication in vivo, leading to suppression of retinitis and improvement in symptoms in CMV colitis (8, 10). DHPG, as a single agent for the treatment of CMV interstitial pneumonitis in bone marrow transplant recipients, demonstrated substantial antiviral activity but did not influence mortality (18). More recently, DHPG in combination with intravenous immune globulin or CMV hyperimmune globulin was shown to significantly reduce CMV-associated morbidity and mortality after bone marrow transplantation compared with DHPG or immune globulin alone (4, 15). With prolonged treatment at therapeutic dosages of 7.5 to 15 mg/kg per day, reversible neutropenia has been observed, frequently necessitating dosage reductions or discontinuation of treatment. Consequent clinical and virological relapses are common (8, 10).

Foscarnet (trisodium phosphonoformate hexahydrate; PFA) is a drug which, like DHPG, inhibits viral DNA polymerase activity, leading to reversible suppression of viral replication (7, 17). In clinical trials in allograft recipients with CMV disease, PFA treatment was associated with suppression of CMV replication and resolution of fever and thrombocytopenia in renal transplant recipients, but not bone marrow transplant recipients, with CMV interstitial pneumonitis (7, 17). PFA has also been reported to lead to resolution of CMV retinitis and interstitial pneumonitis in patients with acquired immunodeficiency syndrome (5, 20). Adverse effects of PFA include anemia, liver enzyme elevations, thrombophlebitis, hypercalcemia, and renal impairment (5, 7, 17, 20). Central nervous system toxicity has been reported with levels in serum exceeding 1 mM (17).

Since toxicity is often a limiting factor in the successful treatment of CMV infections, we were interested in determining whether a combination of DHPG and PFA would act in an additive or synergistic manner, thus lowering the effective concentrations of the drugs required to inhibit CMV replication. Using a plaque reduction assay, we studied the effects of DHPG and PFA both individually and in combination on the in vitro replication of three clinical isolates and the laboratory strain AD-169 of CMV.

Virus cultures were performed in the MRC-5 cell line (American Type Culture Collection, Rockville, Md.). Clinical CMV isolates were used at passage levels four to six. The plaque reduction assay was performed in 24-well tissue culture plates containing confluent MRC-5 cell monolayers. Wells were inoculated with 50 to 150 PFU of the CMV isolates in a 0.1-ml inoculum. After a 60-min adsorption period, the monolayers were washed and then fed with Eagle minimal essential medium with 2% fetal calf serum, and test drugs were added to quadruplicate wells at the following concentrations: 0, 2, 4, 8, 16, or 32 μM DHPG (Syntex Research, Palo Alto, Calif.); 0, 50, 100, 150, 200, or 400 μM PFA (Astra Pharmaceuticals, Sodertalje, Sweden); and combinations of 1, 2, 4, or 8 μM DHPG with 30, 60, or 90 μM PFA. Cultures were maintained at 37°C in a 5% CO2 humidified incubator. The medium was changed at 24 h and every 2 days thereafter with Eagle minimal essential medium containing 2% fetal calf serum and appropriate drug concentrations. On day 10, the monolayers were washed, fixed, stained with 10% formaldehyde-0.02% crystal violet, and air dried. Plaques were counted by light microscopy, and 50% inhibitory concentrations (IC50s) of individual drugs and drug combinations were calculated by the Reed-Muench method (16).

IC50s of the drugs alone or in combination are shown in Table 1. Data are the results of two experiments. Mean IC50s were derived by averaging the percent inhibition data for the individual isolates before the Reed-Muench calculations. The mean IC50 for DHPG alone was 9.0 μM, and for PFA it was 133.0 μM. The IC50 of DMPG for individual clinical isolates ranged from 6.3 to 9.9 μM, and that of PFA ranged between 108.5 and 163.8 μM. For our laboratory strain AD-169, the IC50 of DHPG was 11.8 μM, and that of PFA was 102.6 μM. These values are consistent with the previously reported inhibitory concentrations for both drugs (9,
TABLE 1. In vitro inhibition of CMV replication by DHPG and PFA alone and in combination

<table>
<thead>
<tr>
<th>Isolate</th>
<th>IC₅₀ of PFA (μM)</th>
<th>IC₅₀ of DHPG (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Alone</td>
<td>With a DHPG concn of:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 μM</td>
</tr>
<tr>
<td>AD-169</td>
<td>102.6</td>
<td>60.8</td>
</tr>
<tr>
<td>192</td>
<td>163.8</td>
<td>70.5</td>
</tr>
<tr>
<td>209</td>
<td>108.5</td>
<td>81.9</td>
</tr>
<tr>
<td>295</td>
<td>143.2</td>
<td>75.4</td>
</tr>
<tr>
<td>Mean</td>
<td>133.0</td>
<td>68.7</td>
</tr>
</tbody>
</table>

* Arithmetic mean could not be accurately determined because the IC₅₀ for one CMV strain was less than the lowest PFA concentration tested.

11, 14, 17). There was no correlation between susceptibility to one drug and susceptibility to the other; the two virus strains most susceptible to DHPG were the least susceptible to PFA.

Interactions between DHPG and PFA were evaluated by the fractional inhibitory concentration (FIC) method (3). The FIC for each evaluable drug combination was calculated by the formula

\[
FIC = \frac{IC \text{ of DHPG in combination}}{IC \text{ of DHPG alone}} + \frac{IC \text{ of PFA in combination}}{IC \text{ of PFA alone}}
\]

by using the mean inhibitory values from Table 1. FICs ranged from 0.63 to 0.85, with a mean FIC of 0.72. An FIC of less than 1.0 suggests synergistic interactions, with smaller values representing a more pronounced interaction. Thus, DHPG and PFA in combination showed a moderate synergistic inhibition of CMV replication. When the data were plotted as a dose isobologram (Fig. 1) (1), the curve obtained provided additional evidence that DHPG and PFA synergistically inhibit CMV replication. Neither drug, individually or in combination, suppressed proliferation of the MRC-5 cell substrate at the maximum concentrations used in these experiments (data not shown).

Both DHPG and PFA inhibit CMV DNA synthesis by blocking viral DNA polymerase activity (7, 9, 17). This inhibition is virostatic, and once the drugs are removed, viral replication again occurs. While both drugs act in a similar manner, they have different sites of toxicity. DHPG tends to suppress granulopoiesis. PFA predominantly affects renal function, but it may also cause hepatotoxicity and anemia. This study shows that when DHPG and PFA were used in combination, suppression of viral replication was achieved at lower drug concentrations than when the drugs were used individually. Treatment of CMV infections with drug combinations may result in similar synergistic effects, providing a mechanism for reducing the severity of drug toxicity, allowing prolongation of antiviral therapy, and increasing rates of recovery from CMV disease.

LITERATURE CITED


FIG. 1. Dose isobologram for evaluation of the activity of DHPG and PFA in combination. Concentrations of DHPG and PFA which, in combination, result in 50% inhibition of viral replication are plotted as fractions of the IC₅₀ of each drug when tested alone. The broken line joining the IC₅₀s of the individual drugs represents an isobole which indicates no interaction. The solid line represents the isobol for DHPG-PFA interactions. The bowing of the isobol towards the origin is indicative of a synergistic interaction.