Human Cytomegalovirus Resistance to Deoxyribosylindole Nucleosides Maps to a Transversion Mutation in the Terminase Subunit-Encoding Gene UL89

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Human cytomegalovirus (HCMV) infection can cause severe illnesses, including encephalopathy and mental retardation, in immunocompromised and immunologically immature patients. Current pharmacotherapies for treating systemic HCMV infections include ganciclovir, cidofovir, and foscarnet. However, long-term administration of these agents can result in serious adverse effects (myelosuppression and/or nephrotoxicity) and the development of viral strains with reduced susceptibility to drugs. The deoxyribosylindole (indole) nucleosides demonstrate a 20-fold greater activity in vitro (the drug concentration at which 50% of the number of plaques was reduced with the presence of drug compared to the number in the absence of drug [EC50] = 0.34 μM) than ganciclovir (EC50 = 7.4 μM) without any observed increase in cytotoxicity. Based on structural similarity to the benzimidazole nucleosides, we hypothesize that the indole nucleosides target the HCMV terminase, an enzyme responsible for packaging viral DNA into capsids and cleaving the DNA into genome-length units. To test this hypothesis, an indole nucleoside-resistant HCMV strain was isolated, the open reading frames of the genes that encode the viral terminase were sequenced, and a G766C mutation in exon 1 of UL89 was identified; this mutation resulted in an E256Q change in the amino acid sequence of the corresponding protein. An HCMV wild-type strain, engineered with this mutation to confer resistance, demonstrated an 18-fold decrease in susceptibility to the indole nucleosides (EC50 = 3.1 ± 0.7 μM) compared to that of wild-type virus (EC50 = 0.17 ± 0.04 μM). Interestingly, this mutation did not confer resistance to the benzimidazole nucleosides (EC50 for wild-type HCMV = 0.25 ± 0.04 μM, EC50 for HCMV pUL89 E256Q = 0.23 ± 0.04 μM). We conclude, therefore, that the G766C mutation that results in the E256Q substitution is unique for indole nucleoside resistance and distinct from previously discovered substitutions that confer both indole and benzimidazole nucleoside resistance (D344E and A355T).


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demonstrated that the benzimidazole ribonucleosides inhibit the maturation of the polygenomic concatameric HCMV DNA by interacting with and inhibiting the HCMV terminase (21, 27, 28). In addition, the benzimidazoles demonstrate a toxicity profile more favorable than that of GCV (29). However, these drugs are readily metabolized in vivo to an inactive aglycone metabolite with an estimated half-life of 0.6 h, which is too rapid for these compounds to be considered clinically viable (30).

The deoxyriboosylindole nucleoside UMJD 1896 (Fig. 1), chemically related to benzimidazole nucleosides, may serve as a viable option for HCMV pharmacotherapy due to its potent and selective antiviral activity. It has previously been demonstrated that this indole nucleoside elicits 20-fold greater activity (the drug concentration at which 50% of the number of plaques was reduced with the presence of drug compared to the number in the absence of drug [EC_{50}] = 0.34 μM) than GCV (EC_{50} = 7.4 μM) without any observed increase in cytotoxicity (31). In addition, it has been established that certain indole nucleosides exert an antiviral effect in the replication cycle that is later than that of GCV but similar to that of the benzimidazole nucleosides (31). Furthermore, a benzimidazole nucleoside-resistant HCMV isolate containing both the D344E and A355T substitutions in pUL89 also exhibits decreased antiviral activity. It has previously been demonstrated that the benzimidazole nucleosides inhibit the normal viral maturation process.

**MATERIALS AND METHODS**

**Chemicals.** Deoxyriboosylindole nucleoside UMJD 1896 [3-acetyl-2-bromo-5,6-dichloro-1-(2-deoxy-β-D-ribofuranosyl)indole] and the benzimidazole ribonucleoside 2-bromo-5,6-dichloro-1-(β-D-ribofuranosyl) benzimidazole (BDCRB) (Fig. 1) were synthesized in the laboratory of L. B. Townsend as described previously (31, 33). GCV was kindly provided by Hoffmann-La Roche (Palo Alto, CA).

**Cell culture procedures.** Human foreskin fibroblasts (HFFs) were grown in minimum essential medium (Eagle) [MEM(E)] with Earle’s salts grown in minimum essential medium (Eagle) [MEM(E)] with Earle’s salts supplemented with 10% fetal bovine serum. They were grown at 37°C under a humidified atmosphere of 3% CO2 and 95% air and were used at passages 15 to 21. The medium was changed every 3 to 4 days.

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**Virus strains.** Wild-type (wt) HCMV strain Towne was kindly provided by M. F. Stinski, University of Iowa. A bacterial artificial chromosome (BAC) clone of strain AD169 (AD169rv) was generously provided by U. H. Koszinowski, Ludwig Maximilian University (Munich, Germany) (35). A glutamate codon (GAG) corresponding to amino acid position number 256 in the UL89 open reading frame was mutated to a glutamine codon, CAG, by introducing a C-to-G transversion mutation at nucleotide position 83022 in the sequence with GenBank accession number AC146999.1. A biallelic kanamycin resistance marker (I-SceI–aphAI) was amplified from a plasmid template (pEPkan-S2) by using oligonucleotide primers UL89_E256Q_Fw (5′-GCA GCA AAA GCA GGT GTC GCA GGT GTC GCA GGA AGT CTT CGG CCG CTG CCA CAC CTA GCG GTA ATA ACA GGG TAA TCT ATT T-3′) and UL89_E256Q_Rv (5′-AAC ATG TAG TCG CGG GCG AGG GTG TTG GGG CAG CAG CAG ACC TGC ACC TCT TTG AGC AGC ACC GCC GAT GGT ACA ACC AAT TAA CCC-3′), which had been custom synthesized, and was purified via polyacrylamide gel electrophoresis (PAGE) by Integrated DNA Technologies, Inc. (Coralville, IA). The resulting PCR product was gel purified using a NucleoSpin gel and PCR cleanup kit (Macherey-Nagel, Inc., Bethlehem, PA) and was then electroporated into Escherichia coli strain SGS1826 (a gift of David Knipe, The Rockefeller University, New York, NY) harboring the AD169rv BAC. Single Kanr colonies were picked, and Red recombinase and I-SceI homing endonuclease activities were induced to remove the I-SceI–aphAI cassette. A kanamycin-sensitive colony containing the recombinant BAC, AD169rv UL89 E256Q, was isolated. The overall genetic integrity of the recombinant BAC was confirmed by restriction enzyme digestion analysis (not shown). The presence of the intended single-base-pair mutation and the absence of spurious changes were verified by DNA sequencing (not shown) of the modified region (Genewiz, Inc., South Plainfield, NJ).

**Virus reconstitution.** Infectious virus was reconstituted from BAC DNA as described previously (25, 39, 40) by cotransfection of pp71 and pUL89 DNA-containing plasmid DNA using lipofection (Corning, Inc.) with a total of 1 μg DNA containing 750 ng Bac DNA, 200 ng of pp71 expression plasmid pgS5-pp71 (a gift of Robert Kaleja, University of Wisconsin-Madison, Madison, WI) (41), and 50 ng of the Cre recombinase expression plasmid pCAGGS-nlsCre (a gift of Michael I. Kotlikoff, Cornell Univer-

**HCMV plaque reduction assay.** HFFs were plated in 24-well cluster dishes at 80,000 cells in a total volume of 1 ml of medium per well and incubated overnight. Each plate was infected with 100 PFU. The virus was allowed to absorb for 2 h at 37°C in a CO2 incubator with intermittent rocking of the dishes. Immediately postinfection, the plates were treated with serial dilutions of drug (0.01 to 100 μM). After incubation for 10 days, the plates were stained with crystal violet (0.1% in 20% methanol), and plaques were enumerated using an inverted microscope at 20- to 30-fold magnification, and EC_{50} were calculated from graphing of the data on a semilog plot.

**Selection of indole nucleoside-resistant virus.** HFFs were infected with HCMV Towne at a multiplicity of infection of 0.01 and grown in a 25-cm2 tissue culture flask in the presence of 0.5 μM indole nucleoside 1896 for 2 weeks. Supernatant progeny virus was then passaged in the presence of, first, a 1.0 μM concentration and then a 2.0 μM concentration of indole nucleoside 1896; the duration for each passage was 2 weeks. The resulting virus was further passaged in 2.0 μM indole nucleoside 1896 for a 3-month period and frozen in liquid nitrogen, and the titer was determined. The resulting virus stock was purified using the Klein limiting dilution method (36) and termed 1896r.

**DNA sequencing.** Primers for PCR amplification and DNA sequencing were deduced on the basis of the published sequence of the wild-type Towne strain of HCMV (GenBank accession number FJ616285.1). The terminase subunits encoded by viral genes UL51, UL56, and UL89 were selectively amplified by PCR. Following amplification, the products were run on a 1% agarose gel, extracted, and purified using a QiAquick gel extraction kit (Qiagen, Valencia, CA). The amplified products were sequenced using an Applied Biosystems 3730xl DNA analyzer (ISU DNA Facility, Office of Biotechnology). Sequences were analyzed and aligned using Clustal Omega software (Cambridgeshire, United Kingdom).

**Marker transfer studies.** To transfer the 1896r mutation hypothetically caused to drug resistance to strain AD169, in passant mutagenesis (37, 38) was performed, as described elsewhere (25, 39, 40), on an infectious BAC clone of HCMV strain AD169, AD169rv (a gift of Ulrich Koszinowski, Munich, Germany) (35). A glutamate codon (GAG) corresponding to amino acid position number 256 in the UL89 open reading frame was mutated to a glutamine codon, CAG, by introducing a C-to-G transversion mutation at nucleotide position 83022 of the sequence with GenBank accession number AC146999.1. Biallelic kanamycin resistance marker (I-SceI–aphAI) was amplified from a plasmid template (pEPkan-S2) by using oligonucleotide primers UL89 E256Q_Fw (5′-GCA GCA AAA GCA GGT GTC GCA GGT GTC GCA GGA AGT CTT CGG CCG CTG CCA CAC CTA GCG GTA ATA ACA GGG TAA TCT ATT T-3′) and UL89 E256Q_Rv (5′-AAC ATG TAG TCG CGG GCG AGG GTG TTG GGG CAG CAG CAG ACC TGC ACC TCT TTG AGC AGC ACC GCC GAT GGT ACA ACC AAT TAA CCC-3′), which had been custom synthesized, and was purified via polyacrylamide gel electrophoresis (PAGE) by Integrated DNA Technologies, Inc. (Coralville, IA). The resulting PCR product was gel purified using a NucleoSpin gel and PCR cleanup kit (Macherey-Nagel, Inc., Bethlehem, PA) and was then electroporated into Escherichia coli strain SGS1823 (a gift of David Knipe, The Rockefeller University, New York, NY) harboring the AD169rv BAC. Single Kanr colonies were picked, and Red recombinase and I-SceI homing endonuclease activities were induced to remove the I-SceI–aphAI cassette. A kanamycin-sensitive colony containing the recombinant BAC, AD169rv UL89 E256Q, was isolated. The overall genetic integrity of the recombinant BAC was confirmed by restriction enzyme digestion analysis (not shown). The presence of the intended single-base-pair mutation and the absence of spurious changes were verified by DNA sequencing (not shown) of the modified region (Genewiz, Inc., South Plainfield, NJ).

**HCMV Resistance to the Indole Nucleosides Maps to UL89**

**FIG 1 Structures of 2-bromo-5,6-dichloro-1-(β-D-ribofuranosyl) benzimidazole (BDCRB) and 3-acetyl-2-bromo-5,6-dichloro-1-(2-deoxy-β-D-ribofuranosyl) indole (UMJD 1896, indole nucleoside 1896).**
FIG 2 Initial characterization of an indole nucleoside 1896-resistant HCMV isolate (1896'). HFFs infected with an indole nucleoside 1896-resistant HCMV isolate (open symbols) were subjected to increasing concentrations of either BDCRB (dashed lines, ■ and □) or indole nucleoside 1896 (solid lines, ▲ and △) and compared to HFFs infected with wild-type HCMV (Towne; closed symbols) subjected to the same concentrations of drug. The y axis represents the percentage of plaques compared to the amount for a no-drug control. The results demonstrated that 1896', while resistant to indole nucleoside 1896, does not exhibit cross-resistance to BDCRB or GCV.

DNA sequencing of 1896'. The benzimidazole nucleoside-resistant HCMV isolates reportedly exhibit cross-resistance to indole nucleoside 1896 (31). This resistance was mapped to mutations in UL56 and UL89, two subunits of the viral terminase enzyme (27, 32). In addition to pUL56 and pUL89, pUL51 may be another subunit that comprises the viral terminase enzyme (24). Therefore, the open reading frames of UL51, UL56, and UL89 in 1896' were sequenced. The results demonstrated that both UL51 and UL56 of 1896' contain no mutations that would affect the amino acid sequence of the translated protein. However, UL89 contains a single-base-pair mutation (G to C) at position 766 (G766C), which is located within the first exon of the gene. The mutation would result in a glutamate-to-glutamine change in the amino acid residue at position 256 of the pUL89 protein (E256Q) (Table 2).

RESULTS

Initial characterization of indole nucleoside 1896-resistant HCMV isolate (1896'). To determine whether the HCMV terminase is the target of the deoxyribosylindole nucleosides, wild-type HCMV Towne was grown in the presence of increasing concentrations of indole nucleoside 1896. An isolate (plaque purified and termed 1896') was subjected to increasing concentrations of drug to confirm resistance (Fig. 2; Table 1). As expected, 1896' was approximately 5-fold more resistant to indole nucleoside 1896 (EC_{50} = 2.3 ± 0.8 μM) than wild-type virus (HCMV Towne) (EC_{50} = 0.46 ± 0.17 μM). In addition, 1896' was subjected to increasing concentrations of BDCRB in order to determine if the virus is cross-resistant to this drug. Interestingly, 1896' exhibited no significant change in susceptibility to BDCRB (EC_{50} = 6.0 ± 2.1 μM) compared to that of wild-type virus (HCMV Towne) (EC_{50} = 3.0 ± 1.1 μM). As expected, 1896' did not exhibit any change in susceptibility to GCV (EC_{50} = 8.7 ± 2.1 μM; wild-type HCMV EC_{50} = 10.9 ± 4.5 μM). These results demonstrate that 1896', while resistant to indole nucleoside 1896, does not exhibit cross-resistance to BDCRB or GCV.

Recombinant HCMV pUL89 E256Q exhibits resistance to indole nucleoside 1896 with no change in benzimidazole nucleoside susceptibility. To determine whether the G766C transversion mutation in UL89 was the mutation that confers antiviral drug resistance in 1896' and corroborate the hypothesis that this drug targets the HCMV terminase, the mutation was engineered into wild-type strain AD169, resulting in HCMV pUL89 E256Q. HFFs infected with this engineered virus were exposed to increasing concentrations of GCV, BDCRB, or indole nucleoside 1896 and were compared to HFFs infected with wild-type strain AD169 under the same conditions. As shown in Fig. 3 and Table 1, HCMV pUL89 E256Q was approximately 18-fold more resistant to indole nucleoside 1896 (EC_{50} = 3.1 ± 0.7 μM) than wild-type virus (EC_{50} = 0.17 ± 0.04 μM). Interestingly, and confirming the results obtained with 1896', the recombinant virus did not exhibit any cross-resistance to BDCRB (EC_{50} for wt HCMV AD169 = 0.25 ± 0.04 μM, EC_{50} for HCMV UL89 E256Q = 0.23 ± 0.04 μM). Furthermore, and similar to the findings for 1896', there was no significant change in the susceptibility of HCMV pUL89 E256Q to GCV (EC_{50} = 0.65 ± 0.21 μM) compared to that of wild-type AD169 virus (EC_{50} = 1.5 ± 0.7 μM). We therefore conclude that the G766C mutation in UL89, which results in an E256Q amino acid change in pUL89 and confers deoxyribosylindole nucleoside analog resistance, is distinct from previously identified amino acid substitutions (D344E and A355T) which confer resistance to both indole and benzimidazole nucleosides.

TABLE 1 Antiviral activity of GCV, BDCRB, and indole nucleoside 1896 against 1896' and HCMV UL89 E256Q

<table>
<thead>
<tr>
<th>Antiviral</th>
<th>Comparison of Towne and 1896'</th>
<th>Comparison of AD169 and HCMV UL89 E256Q</th>
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<tbody>
<tr>
<td>EC_{50} (μM)</td>
<td>Fold resistance of 1896'</td>
<td>HCMV UL89 E256Q</td>
</tr>
<tr>
<td>GCV</td>
<td>wt (Towne) 1896'</td>
<td>10.9 ± 4.5 8.7 ± 2.1</td>
</tr>
<tr>
<td>BDCRB</td>
<td>3.0 ± 1.1 6.0 ± 2.1</td>
<td>0.25 ± 0.04 0.23 ± 0.04</td>
</tr>
<tr>
<td>1896</td>
<td>0.46 ± 0.17 2.3 ± 0.8</td>
<td>0.17 ± 0.04 3.1 ± 0.7</td>
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\( a \) P < 0.05.  
\( b \) P < 0.001.
DISCUSSION

The deoxyribosylindole nucleosides elicit an antiviral effect late in the HCMV replication cycle (31). Since it has previously been demonstrated that mutations in the HCMV genome that confer resistance to the chemically related benzimidazole ribonucleosides also confer resistance to the indole nucleosides (31) and that those mutations occur in the open reading frames of the viral terminase enzyme (24, 27, 43), we hypothesized that the molecular mechanism by which the indole nucleosides inhibit viral replication is targeting of the HCMV terminase. Our current results demonstrate that a previously uncharacterized point mutation in UL89, which results in a single amino acid residue change at position 256 of the encoded protein (Table 2), renders the virus resistant to the indole nucleosides but, interestingly, not to the benzimidazole nucleoside BDCRB (Table 1). These results establish that pUL89 is a target of the indole nucleosides.

Previous studies have demonstrated that certain mutations can decrease the replication capacity of virus upon infection (39, 44). However, mutations that confer resistance to the chemically related benzimidazole ribonucleosides also confer resistance to the indole nucleosides (31) and that those mutations occur in the open reading frames of the viral terminase enzyme (24, 27, 43), we hypothesized that the molecular mechanism by which the indole nucleosides inhibit viral replication is targeting the HCMV terminase. Our current results demonstrate that a previously uncharacterized point mutation in UL89, which results in a single amino acid residue change at position 256 of the encoded protein (Table 2), renders the virus resistant to the indole nucleosides but, interestingly, not to the benzimidazole nucleoside BDCRB (Table 1). These results establish that pUL89 is a target of the indole nucleosides.

Previous studies have demonstrated that certain mutations can decrease the replication capacity of virus upon infection (39, 44). However, mutations that confer resistance to the chemically related benzimidazole ribonucleosides did not affect the replicative fitness of those viruses (27). Although the replicative fitness of the viruses presented herein was not directly compared, wild-type virus engineered with the G766C mutation in UL89 (HCMV pUL89 E256Q) replicated to titers that were indistinguishable from those observed for wild-type AD169 HCMV (data not shown). This general observation leads us to speculate that viral fitness was not reduced due to the introduction of the G766C mutation that confers resistance to indole nucleoside 1896.

GCV and cidofovir have been found to elicit an antiviral effect through direct inhibition of the viral DNA polymerase and/or incorporation into elongating viral DNA, resulting in chain termination (7). Therefore, it is not surprising that many mutations in the viral DNA polymerase that result in resistance to one of these drugs also result in decreased susceptibility to the other (45).

TABLE 2

<table>
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<tr>
<th>Amino acid sequence of pUL89</th>
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<tr>
<td>HCMV strain</td>
</tr>
<tr>
<td>Towne</td>
</tr>
<tr>
<td>AD169</td>
</tr>
<tr>
<td>1038rB</td>
</tr>
<tr>
<td>1896r</td>
</tr>
<tr>
<td>E256Q</td>
</tr>
</tbody>
</table>

a The amino acid sequence of pUL89 was inferred from the UL89 open reading frame. Only the portion of the sequence which contained the substitutions that confer resistance is presented. Amino acid changes are shown in boldface.

b A BDCRB-resistant HCMV isolate containing both D344E and A355T.

c A recombinant HCMV isolate containing the G766C mutation in UL89.
Since it has been previously established that mutations that result in the resistance of HCMV to the benzimidazole nucleosides confer resistance to the indole nucleosides (31), the implication is that these compounds elicit an antiviral effect in a similar manner. As such, we contend that the most likely mechanism of action for the indole nucleosides is inhibition of pUL89 nuclease activity, particularly because the benzimidazole nucleosides inhibit HCMV terminase function in that manner (21). In addition, it has also been established that the benzimidazole nucleosides inhibit the interaction of pUL56 with the viral portal protein (pUL104), thereby preventing insertion of the viral genome into the procapsid (28). Therefore, it seems likely that the indole nucleosides would also inhibit the insertion of the viral genome into the capsid by preventing the interaction of pUL56 and pUL104. Nonetheless, while pUL104 does associate with the viral terminase for processing and packaging and previous studies have demonstrated there is a mutation in UL104 common in benzimidazole-resistant HCMV isolates (46), this mutation does not in and of itself confer resistance. Thus, we did not sequence UL104 in 1896, especially since the mutation discovered in UL89 was sufficient for drug resistance.

Despite their many similarities, however, there are important differences between the benzimidazole and indole nucleosides. We hypothesize that key chemical differences found in the indole nucleosides but not the benzimidazole nucleosides confer enough structural dissimilarity between the two compounds to allow them to bind their targets in a similar but not precisely the same manner. Chemically, they differ by the presence of an acetyl group at the 3 position of the indole ring, the lack of nitrogen at the 3 position of the indole compared to the benzimidazole, and the lack of a hydroxyl group at the 2’ position of the sugar (31). Our results presented herein demonstrating that a mutation in pUL89 that confers resistance to the indole nucleosides but not the benzimidazole nucleosides provides evidence for this hypothesis (Table 1). In addition, we speculate that these key chemical differences that allow the indole nucleosides to bind to the viral terminase in a different manner may also allow in vivo stability greater than what has been observed with the benzimidazole nucleosides. Although previous studies have demonstrated that the benzimidazole nucleosides elicit good antiviral activity and selectivity in vitro (33), they are metabolized via glycosidic bond degradation too rapidly in vivo to be considered viable clinical candidates (30). The chemical differences between the indole and the benzimidazole nucleosides result in a stronger glycosidic bond, and we therefore hypothesize that the indole nucleosides will not be as readily metabolized in vivo (31). Further investigation into the metabolism of the indole nucleosides is warranted if these compounds are to be considered viable for clinical use.

Current pharmacotherapies (GCV, cidofovir, foscarnet) and some currently in clinical trials (brincidofovir, cyclopropavir) for the treatment of systemic HCMV infections target the viral DNA polymerase (pUL54). Previous studies have demonstrated high levels of cross-resistance between these compounds (47–49). Since long-term pharmacotherapy is generally required due to the recurrence of infection upon cessation of treatment, the development of HCMV strains with reduced susceptibility to multiple drugs is a major concern. In addition to the increased safety profile of the indole nucleosides, the main advantage to this pharmacotherapy is the distinct mechanism of action by which this compound elicits an antiviral effect. As such, no significant levels of cross-resistance should exist between the indole nucleosides and other pharmacotherapies that target the viral DNA polymerase. Consistent with this is our results presented herein demonstrating no significant change in the susceptibility of mutant (HCMV pUL89 E256Q) virus to GCV compared to that of wild-type AD169 virus subjected to the same concentrations of drug (Table 1).

Interest in the HCMV terminase, comprised of subunits pUL51, pUL56, and pUL89, as a potential target for antiviral pharmacotherapy is increasing (24, 25). In addition to the benzimidazole and the indole nucleosides, BAY 38-4766, iberimovir, and the anti-HIV integrase inhibitor raltegravir are a few compounds that elicit an antiviral effect against HCMV through inhibition of the terminase function (50–52). Although it appears that all these compounds affect the terminase by different and distinct mechanisms, the result is the same: impairment of viral genome processing and packaging. While we consider it unlikely that any significant levels of cross-resistance between currently approved pharmacotherapies and the indole nucleosides would occur, it remains plausible that mutations conferring resistance to the indole nucleosides could confer cross-resistance to other compounds that target the HCMV terminase. This possibility, however, was not addressed in the present study.

With the use of monotherapy for the treatment of HCMV infections, the development of drug resistance (and possibly cross-resistance) can occur within 2 weeks of initiation of therapy (17). It is therefore important to develop new pharmacotherapies that have not only a greater safety profile but also a different mechanism of action so as to minimize the possibility of drug resistance and/or cross-resistance. Previous results have demonstrated that the indole nucleosides have greater antiviral activity than GCV without any observed increase in cytotoxicity (31). Our results presented herein demonstrate a distinct mechanism of action for the indole nucleosides compared to the mechanisms of action of currently approved pharmacotherapies. Further development and examination of the molecular mechanism of action of these compounds are warranted.

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REFERENCES


Evers DL, Komazin G, Ptak RG, Shin D, Emmer BT, Townsend LB, Drach JC. 2004. Inhibition of human cytomegalovirus replication by benzimidazole nucleosides involves three distinct mechanisms. Antimi-


