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Ganciclovir-Resistant Cytomegalovirus Clinical Isolates: Mode of Resistance to Ganciclovir

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Cytomegalovirus strains with reduced in vitro susceptibilities to ganciclovir have been recovered from patients who failed long-term ganciclovir therapy. The ganciclovir-resistant clinical isolates in this study were unable to induce ganciclovir phosphorylation in virus-infected cells. The viral DNA polymerase function appeared unaltered in one genetically pure ganciclovir-resistant strain, compared with that of its wild-type ganciclovir-sensitive counterpart. All nine of the ganciclovir-resistant strains were susceptible to foscarnet. Moreover, these strains were sensitive to inhibition both by vidarabine and 1-(2′-deoxy-2′-fluoro-β-D-arabinofuranosyl)-5-iodocytosine (FIAC), antiviral agents that are activated by cellular enzymes, and by (S)-1(3-hydroxy-2-phosphonylmethoxypropyl)cytosine (HPMPC), which is a monophosphate nucleoside analog. The in vitro resistance to ganciclovir of the ganciclovir-resistant clinical isolates studied was attributed to the inability of the cells infected with these isolates to phosphorylate ganciclovir; the virally encoded DNA polymerase did not appear to play a role in this ganciclovir resistance.

Human cytomegalovirus (CMV) infection is a serious complication in immunocompromised individuals and is a major cause of morbidity and mortality (35, 36, 45). In bone marrow and solid organ transplant recipients, CMV is often associated with interstitial pneumonia (33, 44). CMV retinitis occurs in up to 20% of people with AIDS (12), and disseminated CMV can be demonstrated at autopsy for over 90% of patients with AIDS (31). AIDS patients and transplant patients also may shed multiple strains of CMV either concurrently or sequentially (14). Treatment with ganciclovir (GCV) is the current therapy of choice for CMV retinitis (9). This disease requires aggressive management, which usually means continuous GCV therapy until the death of the patient (23, 34).

A previous report described the clinical courses of one chronic lymphocytic leukemia patient and two AIDS patients who received extended GCV therapy for severe CMV disease and who ultimately failed to respond to therapy. In vitro GCV resistance was demonstrated in CMV isolated from these patients—in one patient, before GCV therapy was begun, and in the other two patients, after prolonged therapy with GCV (15). On the basis of currently available data from GCV clinical trials, approximately 8% of AIDS patients with CMV retinitis who receive long-term maintenance therapy develop GCV-resistant strains of CMV (13). We have examined both the antiviral drug profiles of the GCV-resistant isolates from eight patients and the biochemical basis for the resistance of these isolates to GCV.

MATERIALS AND METHODS

Cells. Human diploid embryonic lung fibroblasts (MRC-5) were obtained either from the American Type Culture Collection (Rockville, Md.) or from Whittaker Bioproducts (Walkersville, Md.) and were used between passages 22 and 26 and passages 27 and 30, respectively. Monolayer cultures were grown in Eagle minimal essential medium containing 50 U of penicillin per ml and 50 μg of streptomycin per ml and supplemented with 8% fetal bovine serum (Sterile Systems, Logan, Utah) and 1% L-glutamine.

Virus. CMV strain AD169 was obtained from the American Type Culture Collection. A laboratory-selected GCV-resistant mutant of AD169, 759^r D100-1, was obtained from D. M. Coen (Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School) (3).

Two sets of CMV clinical isolates were examined. The first set consisted of seven clinical isolates recovered from three patients treated at the University of Minnesota Health Sciences Center in Minneapolis (MN set). The clinical courses of these patients have been described, and, except for C8805, the GCV susceptibilities of these isolates have already been reported (15). C8801 (pretherapy) and C8702 (late therapy), both GCV-resistant virus strains, are original mixed isolates from patient 1. C8708 (GCV-sensitive [early therapy]) and C8704 (GCV-resistant [late therapy]) isolates are from patient 2. C8705 (GCV-sensitive [early therapy]), C8805 (GCV-resistant [midtherapy]), and C8706 (GCV-resistant [late therapy]) isolates are from patient 3. These isolates have been plaque purified by using cell-free virus. Each number following an isolate designation indicates a single plaque purification; C8705-3 has been plaque purified once, and C8708 17-1-1 has been plaque purified three times.

Seven additional clinical isolates were recovered from five AIDS patients being treated at the Mt. Zion Hospital and Medical Center in San Francisco (ZN set). C8915, C8916, C8917, and C8918 are GCV-resistant isolates from the blood of four different patients. C8912, C8913, and C8914 are early-, mid-, and late-therapy isolates from the semen of one patient, and these sequential isolates exhibit decreasing levels of susceptibility to GCV. These isolates have not been plaque purified but have been maintained as low-passage mixed populations.

Both mixed-population and plaque-purified clinical-isolate virus stocks were maintained as cell-associated virus. Cells

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were infected by inoculating established monolayers with a defined number of viable, infected, plaque-forming cells. Laboratory strains of CMV were maintained and used as cell-free virus stocks.

Antiviral drugs and candidate antiviral compounds. GCV and acyclovir (ACV) were synthesized at Burroughs Wellcome Co. (Research Triangle Park, N.C.) laboratories; vidarabine (ARAA) was obtained from Parke-Davis Co. (Morris Plains, N.J.); foscarnet (PFA) was obtained from Fluka AG Chemicals Fabrik (Buchs, Switzerland). Other antiviral agents were obtained as follows: (*S*)-1(3-hydroxy-2-phosphonylmethoxypropyl)cytosine (HPMPC) was kindly provided by John Martin of Bristol-Myers Co. (Wallingford, Conn.); 1-(2′-deoxy-2′-fluoro-β-D-arabinofuranosyl)-5-iodocytosine (FIAC) came from Oclassen Pharmaceuticals, Inc. (San Rafael, Calif.).

Antiviral drug susceptibility assays. Susceptibilities to antiviral compounds were measured by either or both of two methods.

(i) Plaque reduction assay. The procedure for this assay (4) was modified to accommodate cell-associated virus. Briefly, cell monolayers in 12-well plates were infected with clinical-isolate virus by inoculation with 50 to 100 plaque-forming cells per well. Cells were allowed to attach for 2 h. Infections with laboratory virus were carried out as described previously (4). Overlays with the appropriate drug concentrations contained 0.4% agarose and 4% fetal bovine serum. In ARAA susceptibility assays, 10 μM *erythro*-9-(2-hydroxy-3-nonyl) adenine hydrochloride (EHNA; Burroughs Wellcome Co.) was included in the overlay medium to inhibit cellular adenosine deaminase. Assay cultures were incubated for 6 to 12 days according to the growth characteristics of the particular virus strain to ensure equivalent plaque development.

(ii) Viral DNA replication assay. A previously described DNA-DNA probe hybridization assay (Diagnostic Hybrids, Inc., Athens, Ohio) was used to measure reduction of viral DNA synthesis (11).

At least seven drug concentrations spanning the linear portion of the susceptibility curve and one virus control were employed in triplicate in all assays, and results were analyzed by regression analysis (SAS Probit procedure, version 82.4; SAS Institute, Cary, N.C.). The antiviral 50% effective doses (ED_{50}s) derived from the DNA replication assay are usually lower than those obtained by plaque reduction assay, perhaps because the DNA replication assay measures viral growth per infected cell, whereas plaque reduction assays measure plaquing efficiency in the presence of drug.

GCV intracellular anabolism assay. Cell monolayers in 60-mm dishes were infected with either 6.5×10^5 to 13×10^5 plaque forming cells of clinically derived virus strains or 4×10^5 to 6.5×10^5 PFU of laboratory strain virus. Both AD169 and the laboratory-selected GCV-resistant mutant, 759^r D100-1, were included in every assay. This mutant has been shown to be deficient in its ability to generate GCV nucleotides in infected cells (3). Infections were allowed to progress for 3 or 4 days, the length of time needed to achieve the maximal rate of GCV phosphorylation in infected cells (4). Cells were then pulse-labeled for the indicated times with 25 to 50 μM 8-^{14}C-GCV (specific activity, 52 mCi/mmol), which had been purified by high-pressure liquid chromatography to remove guanine contaminants. GCV anabolites were measured with a cation-exchange column according to a previously described method (17).

CMV strain identification. Strain identities were determined by comparing DNA restriction endonuclease patterns. Total intracellular DNA from virus-infected cells was extracted by a previously described method (19), digested with restriction enzymes (*Eco*RI, *Bam*HI, or *Hin*dIII), and analyzed by electrophoresis through 0.8% agarose gels. Southern blot transfers were probed with ^{32}P-labeled CMV DNA which had been purified on CsCl gradients after extraction from sucrose gradient-purified virions. Purified CMV DNA was provided by E.-S. Huang (Lineberger Cancer Center, University of North Carolina, Chapel Hill). The DNA was then labeled with ^{32}P by standard nick translation methods.

DNA polymerase purification and drug inhibition assays. CMV DNA polymerase and HeLa DNA polymerase α were partially purified from nuclear extracts through DEAE-cellulose by published procedures (21). HeLa DNA polymerase α was further purified by phosphocellulose and DNA cellulose chromatography (21, 28).

Polymerase α reaction mixtures contained 50 mM Tris-HCl (pH 7.5), 6 mM $MgCl_2$, 50 μg of bovine serum albumin (BSA), 1 mM dithiothreitol, 200 μg of activated calf thymus DNA, 50 μM dATP, 50 μM dCTP, 50 μM dTTP, 10 μM [^{3}H]dGTP (0.5 μCi/nmol), enzyme (0.5 U), and inhibitor in a total volume of 100 μl. CMV DNA polymerase reaction mixtures contained 50 mM Tris-HCl (pH 8.5), 100 mM $(NH_4)_2SO_4$, 12 mM $MgCl_2$, 50 μg of BSA, 1 mM DTT, 200 μg of activated calf thymus DNA, 50 μM dATP, 50 μM dCTP, 50 μM dTTP, 10 μM [^{3}H]dGTP (0.5 μCi/nmol), enzyme (0.5 U), and inhibitor in a total volume of 100 μl. For the analysis of PFA inhibition, the concentrations of dATP, dCTP, and dTTP were reduced to 10 μM. The reaction mixtures were equilibrated at 37°C, and the reactions were initiated by the addition of enzyme. Samples (15 μl) were removed at five time points and spotted on 4-cm^2 squares of DE81 paper. The paper was washed five times for 5 min each time with 5% Na_2HPO_4, once with water, and twice with 95% ethanol, dried, and counted in Ready Safe liquid scintillation fluid.

RESULTS

Strain identification. The strain identities of the MN set of isolates have been previously reported; the two isolates from patient 1 were identical, the two isolates from patient 2 were identical, and two isolates from patient 3 were different (15). We have since acquired an additional (midtherapy) isolate (C8805) from patient 3 and have determined by restriction enzyme analysis and Southern blotting that this GCV-resistant isolate appears to be nearly identical to the first isolate. Patient 1 was infected with a strain that was already GCV resistant prior to actual GCV therapy. Patients 2 and 3 both harbored GCV-susceptible virus in early therapy and GCV-resistant virus after 131 and 113 days of therapy, respectively. A second strain of CMV was isolated from patient 3 late in the course of his disease; this second strain was also GCV resistant when it was recovered. Serial isolates recovered from the semen of a single patient in the ZN set (C8912, C8913, and C8914) have also been subjected to restriction enzyme analysis. The restriction patterns for these three isolates, although not strictly identical, have differences that are too minor to justify classification of these isolates as different strains.

Anabolism of GCV. Since GCV has been shown to be preferentially anabolized in CMV-infected cells (4), we looked at the intracellular levels of GCV nucleotides in cells infected with clinical isolates. GCV-resistant clinical isolates in both groups showed reduced abilities to induce phosphor-

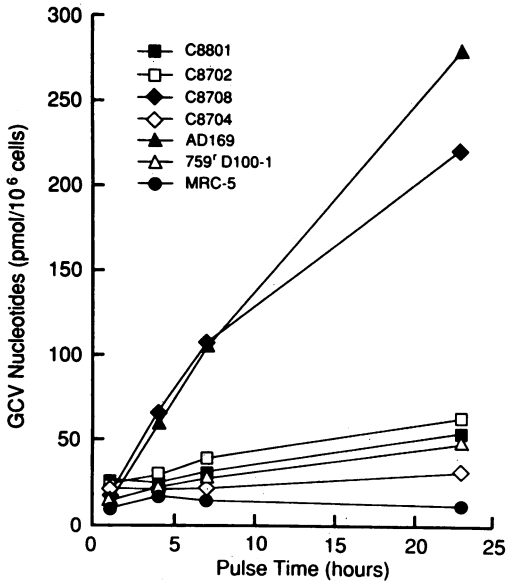

FIG. 1. Intracellular anabolism of GCV by MN set clinical isolates. The patient 1 pretherapy (C8801) and late-therapy (C8702) isolates were both resistant to GCV in vitro. The patient 2 early-therapy isolate (C8708) was susceptible, and the patient 2 late-therapy isolate (C8704) was resistant to GCV in vitro. 759^r D100-1 is a GCV-resistant mutant of AD169. Uninfected MRC-5 cells were included as a control. Infected and uninfected cells were pulse-labeled on day 3 with 50 μM ^{14}C-GCV for indicated times. Anabolites were measured with a cation-exchange column.

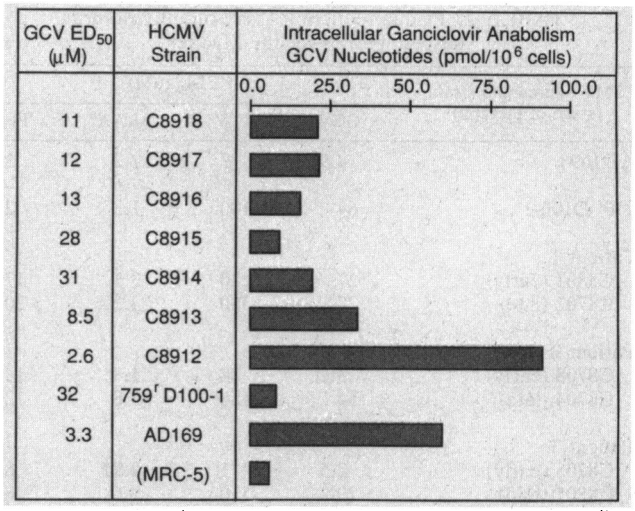

FIG. 2. Intracellular GCV anabolisms of ZN set clinical isolates paired with in vitro susceptibilities to GCV. Infected and uninfected cells were pulse-labeled on day 4 with 25 μM ^{14}C-GCV for 16 h. Anabolites were measured with a cation-exchange column. GCV susceptibilities were determined by a DNA-DNA probe hybridization assay.

ylation of GCV, whereas GCV-susceptible clinical isolates were able to induce phosphorylation at levels comparable to those of the laboratory strain AD169.

Both patient 1 isolates—pretherapy (C8801) and posttherapy (C8702)—were GCV resistant and were also unable to anabolize GCV (Fig. 1). The GCV-susceptible early-therapy isolate (C8708) from patient 2 did anabolize GCV, whereas the GCV-resistant late-therapy isolate (C8704) did not. Results for the MN set patient 3 isolates (data not shown) were similar; i.e., the GCV-susceptible isolate (C8705) induced the formation of GCV nucleotides, whereas the two GCV-resistant isolates (C8706 and C8805) did not.

Levels of phosphorylated GCV induced in infected cells by the ZN set isolates have been paired with the ED_{50}s of GCV for those isolates (Fig. 2). The decreasing GCV susceptibilities demonstrated by early-, mid-, and late-therapy isolates (C8912, C8913, and C8914) from one patient in this group of isolates are mirrored by the decreasing abilities to induce GCV phosphorylation and illustrate a shift from susceptible to resistant virus among mixed-population isolates.

CMV polymerase and HeLa polymerase α analysis. The partially purified CMV DNA polymerases from the isolates of patient 2 (MN set) were examined to determine whether the polymerases played a role in the GCV resistance of the posttherapy isolates. First, homogeneous virus populations were derived from the pretherapy (C8708) and posttherapy (C8704) isolates by three cycles of plaque purification (C8708 17-1-1 and C8704 9-4-1). The GCV susceptibilities of these two resulting strains were consistent with those of the parent populations. The GCV-anabolism phenotypes of the parent isolates were also preserved in these clones (data not shown). The viral DNA polymerases, which were partially purified from the extracts of cells infected with these strains, appeared authentic by the criteria of the salt stimulation and synthetic template utilization which are characteristic of the DNA polymerases encoded by the herpesviruses (21). The viral and cellular enzyme preparations exhibited K_m values for dGTP similar to those previously reported (41) (Table 1). Furthermore, the expected inhibition of such partially purified HeLa polymerase α and AD169 DNA polymerase by the triphosphates of both ACV and GCV was observed (5). Sensitivity to PFA as a PP_i analog which directly inhibits polymerase activity is another potential indicator of changes in viral DNA polymerase. The DNA polymerases of both

TABLE 1. CMV polymerase and HeLa polymerase α analysis

Type of DNA polymerase	GCV phenotype[a]	Inhibition constants (μM) ± SD[c]		
		Inhibitor[b]	K_i	K_m dGTP
HeLa α		ACVTP	0.57 ± 0.06	0.51 ± 0.04
AD 169	S	ACVTP	0.023 ± 0.004	1.1 ± 0.1
C8708 17-1-1	S	ACVTP	0.030 ± 0.003	0.79 ± 0.05
C8704 9-4-1	R	ACVTP	0.017 ± 0.002	0.83 ± 0.08
HeLa α		GCVTP	16 ± 1.8	
AD169	S	GCVTP	0.55 ± 0.06	
C8708 17-1-1	S	GCVTP	0.31 ± 0.04	
C8704 9-4-1	R	GCVTP	0.28 ± 0.04	
HeLa α		PFA	17.5 ± 0.73[d]	
AD169	S	PFA	4.4 ± 1.1[d]	
C8708 17-1-1	S	PFA	3.3 ± 0.23[d]	
C8704 9-4-1	R	PFA	3.6 ± 0.51[d]	

[a] S, susceptible; R, resistant.
[b] TP, triphosphate.
[c] K_i values were obtained from linear regression analysis of the reciprocal of the initial rate versus inhibitor concentration (Dixon plots) where the x-intercept = $K_i[1 + (S/K_m)]$.
[d] Apparent K_i value obtained at 10 μM each dATP, dCTP, dTTP, and dGTP.

TABLE 2. Plaque reduction assay of antiviral drug susceptibilities for the MN set[a]

CMV source and strain (stage of therapy)	ED_{50} (μM)			
	GCV	ACV	ARAA[b]	PFA
AD169	4.9	72	2.2	31
759[r] D100-1	64	100	1.3	24
Patient 1				
C8801 (early)	27	220	3.1	170
C8702 (late)	27	380	4.1	200
Patient 2				
C8708 (early)	3.2	88	1.8	140
C8704 (late)	54	180	5.8	120
Patient 3				
C8705 (early)	2.5	71	0.91	83
C8805 (mid)	29	510	2.6	80
Patient 3, C8706 (late)	19	160	3.1	150

[a] Drug susceptibilities were measured by plaque reduction assay with MRC-5 cells. Seven drug concentrations and virus controls were tested in triplicate, and data were analyzed by SAS Probit. The assays were repeated two or more times. Data are derived from a representative assay. Only data with a standard deviation of ≤20% were considered. Isolate sets were always assayed simultaneously.

[b] A 10 μM concentration of adenosine deaminase inhibitor EHNA was included in the assay medium.

TABLE 3. Hybridization assay of antiviral drug susceptibilities[a]

CMV source and strain (stage of therapy)	ED_{50} (μM)					
	GCV	ACV	ARAA[b]	PFA	HPMPC	FIAC
AD169	1.7	52	0.65	50	0.49	1.1
759[r] D100-1	39	130	0.89	35	1.5	1.3
MN patient 1						
C8702 5-3-1 (late)	13	200	1.1	150	0.32	4.1
MN patient 2						
C8708 17-1-1 (early)	2.1	120	1.8	250	0.90	0.83
C8704 9-4-1 (late)	19	190	2.1	150	1.1	0.56
MN patient 3						
C8705-3 (early)	1.3	70	2.1	110	0.69	1.1
C8805-37 (mid)	12	160	0.60	62	0.81	0.73
MN patient 3, C8706-13 (late)	6.2	200	1.2	150	0.79	0.66
ZN patient 1						
C8912 (early)	1.8	140	2.1	330	2.8	1.0
C8914 (late)	21	440	2.7	320	2.9	1.1
ZN patient 2, C8915	21	95	2.0	220	1.1	1.4
ZN patient 3, C8916	17	89	7.5	120	0.24	4.3
ZN patient 4, C8917	13	260	2.8	180	0.91	0.23
ZN patient 5, C8918	12	190	2.6	240	0.27	0.32

[a] Drug susceptibilities were measured by DNA-DNA probe hybridization assay with MRC-5 cells. Seven drug concentrations and virus controls were tested in triplicate in each assay, and data were analyzed by SAS Probit. The assays were repeated two or more times. Data are derived from a representative assay. Only data with a standard deviation of ≤20% were considered. Isolate sets were always assayed simultaneously.

[b] A 10 μM concentration of adenosine deaminase inhibitor EHNA was included in the assay medium.

C8708 17-1-1 (early-therapy) and C8704 9-4-1 (late-therapy) isolates show comparable sensitivities to the triphosphates of GCV and ACV and to PFA. Moreover, the inhibition values are similar to those measured for the AD169 laboratory strain. Therefore, there is no correlation between in vitro GCV resistance of C8704 9-4-1 and reduced sensitivity of its viral DNA polymerase to the triphosphate form of GCV.

Antiviral susceptibility profiles. The isolate populations from the MN set patients were initially analyzed for in vitro susceptibilities to several well-characterized antiviral agents by the plaque reduction assay (Table 2). The plaque-purified virus strains that were subsequently derived from these populations were then examined for in vitro antiviral susceptibilities by the DNA hybridization assay. The mixed populations from the ZN set of isolates were also tested by the hybridization method (Table 3). The GCV susceptibility values for the MN set strains maintained similar relative ratios between the two assays, although the absolute values derived from the DNA hybridization assay were often lower than those measured by the standard plaque reduction assay. The paired isolates exhibit a trend of marginal cross-resistance to ACV for the posttherapy GCV-resistant strain of each pair (up to a threefold increase in ACV ED_{50}).

The susceptibilities of these isolates to PFA were within the ranges observed for a variety of other clinical isolates and laboratory strains (18). Variations in the susceptibilities of these isolates to PFA are not consistent with GCV resistance. The difference in ED_{50}s between the two late-therapy isolates of patient 3 (MN set) can be attributed to a change in strain. Original low-passage virus of the late-therapy GCV-resistant isolates from patients 2 (C8708) and 3 (C8805 and C8706) consistently demonstrated cross-resistance to ARAA. This may represent a fraction of the isolate population with a DNA polymerase alteration. The plaque-purified viruses of these isolates, C8708 9-4-1, C8805-37, and C8706-13, retain the GCV resistance phenotype and are unable to induce phosphorylation of GCV in infected cells but do not exhibit resistance to ARAA.

The clinical strains showed considerable variation in their in vitro susceptibilities to FIAC and to HPMPC. The range of susceptibilities to FIAC for the GCV-resistant strains was 0.23 to 4.3 μM (mean = 1.5 μM; median = 0.73 μM); for GCV-susceptible strains, the range was 0.83 to 1.1 μM (mean = 0.98 μM; median = 1.0 μM). The range of susceptibilities to HPMPC of the GCV-resistant strains was 0.24 to 2.9 μM (mean = 0.94 μM; median = 0.81 μM), which is similar to the range, 0.69 to 2.8 μM (mean = 1.5 μM; median = 0.90 μM), observed for the three GCV-susceptible clinical strains.

Changes in susceptibilities to FIAC or to HPMPC in the three isolate pairs were not associated with shifts in their GCV susceptibilities. On the other hand, the GCV-resistant laboratory mutant, 759[r] D100-1, was an exception with a reproducible three- to sixfold shift in HPMPC ED_{50}s. Since HPMPC targets the DNA polymerase directly, these results are consistent with the presence of an additional mutation in the laboratory strain, one which resides in the DNA polymerase gene (see Discussion).

DISCUSSION

The management of CMV infections in immunocompromised patients remains a major clinical challenge. Although ACV has been shown to be efficacious for the prophylactic treatment of transplant patients (1), it is not effective in the treatment of active CMV disease. GCV is the current therapy of choice for active CMV retinitis in AIDS patients (9, 23, 34).

As with ACV, it is the triphosphate of GCV that selectively inhibits the viral DNA polymerase (32). Both ACV and GCV are converted to the monophosphate forms by the virus-encoded thymidine kinase (TK) of herpes simplex virus (HSV) and of varicella zoster virus (VZV) and subsequently to the triphosphate forms by cellular enzymes. In contrast to HSV and VZV, CMV does not encode a viral TK (16). Although several attempts have been made to identify cellular enzymes that might be responsible for the initial phosphorylation of GCV, evidence for their role as the principal GCV-phosphorylating enzymes in CMV-infected cells is lacking (25, 40). GCV triphosphate levels increase with increasing concentrations of drug in CMV-infected cells but not in uninfected cells (4). Cells infected with the GCV-resistant mutant, 759^r D100-1, have been shown to take up GCV but do not efficiently phosphorylate the drug (3). These data provide evidence that GCV is selectively monophosphorylated by either a virus-encoded or a virally mediated cellular enzyme. The identity of this enzyme is being actively investigated in this laboratory.

The closely related nucleoside analog, ACV, appears to produce a wide range of ED_{50}s in these clinical isolates. However, there is a weak but consistent trend of reduced susceptibility of the GCV-resistant posttherapy isolates to ACV. These data suggest that ACV is a weak substrate for the GCV-phosphorylating function. This is consistent with the observation that the pretherapy isolate from patient 1 was already GCV resistant. This patient had received extensive ACV therapy for HSV infection prior to the isolation of this GCV-resistant virus (15).

The susceptibilities of these GCV-resistant clinical isolates to the other antiviral nucleosides indicate that the GCV anabolism function is not essential to the antiviral activity of these compounds. There is no cross-resistance of the GCV-resistant isolates to HPMPC or FIAC. HPMPC, as a monophosphate nucleoside analog, does not require the initial virus-induced phosphorylation required by GCV. FIAC is known to be a substrate for phosphorylation by host nucleoside kinases (7, 8). ARAA is phosphorylated by host deoxycytidine and adenosine kinases (22, 29). Although there are no notable differences between pre- and posttherapy isolates in their susceptibilities to these compounds, the observed strain variations in susceptibilities to these compounds provide evidence that a substantial heterogeneity of the virus-encoded DNA polymerase gene exists among clinical strains of CMV.

The patterns of resistance for GCV-resistant clinical isolates described in this report are similar to those described for the laboratory-derived GCV-resistant mutant 759^r D100-1. In CMV isolates recovered from patients who fail to respond to GCV therapy, there is a direct association of in vitro GCV resistance with the inabilities of these isolates to induce GCV phosphorylation in infected cells. Our data show a strong inverse relationship between the in vitro GCV susceptibility of an isolate and the ultimate levels of phosphorylated forms of GCV detected in the virus-infected cells.

However, GCV resistance due to changes in the viral polymerase could not be ruled out solely on the basis of the phenotypic profile of virus strains with regard to the polymerase inhibitor PFA. This is significant because the laboratory mutant, 759^r D100-1, was recently shown to contain two independent mutations; one of these mutations maps directly to the DNA polymerase gene and does not affect intracellular GCV anabolism (42). This polymerase mutation is responsible for the reduced susceptibility of 759^r D100-1 to HPMPC, compared with the parent virus, AD169 (Table 3), but it produces no change in PFA susceptibility and, in the recombinant polymerase mutant, only three- to fivefold-higher GCV ED_{50}s. Changes in the in vitro susceptibilities of HSV to ACV and to GCV have resulted from laboratory-induced mutations in the viral DNA polymerase gene. These virus mutants may exhibit only marginal reductions in ACV or GCV susceptibility, which do not always correlate with similar reductions in sensitivity to PFA (20, 27, 30). It is noteworthy that viral DNA polymerase mutants of both HSV (10, 38) and VZV (2) have been recovered from immunosuppressed patients after prolonged therapy with ACV. To examine the role of an altered DNA polymerase gene that might contribute to GCV resistance in these isolates, we looked at the polymerases of a susceptible virus and a resistant virus from one patient. The partially purified viral DNA polymerases of resistant and susceptible MN set patient 2 strain viruses showed no differences in sensitivity to inhibition by the triphosphates of GCV or ACV or PFA.

With the continuing specter of antiviral drug resistance in the highly immunocompromised population, it would be useful to monitor in vitro antiviral susceptibilities of all the herpesviruses during prolonged antiviral therapy. PFA may offer a therapeutic alternative in cases of GCV-resistant virus infections or in cases of patient intolerance of GCV. Early studies with small numbers of patients indicate that PFA is effective for the treatment of CMV disease (24, 26, 37, 43). PFA has the advantage that it can be administered concurrently with zidovudine, since the adverse events of PFA are primarily renal and metabolic rather than hematologic (24, 26, 37, 43). A treatment strategy of alternating therapies that have different modes of action could, theoretically, circumvent therapeutic failures due to the selection of drug-resistant virus. The reemergence of predominantly TK-competent virus in isolates from patients who previously shed ACV-resistant, TK-deficient virus after cessation of ACV therapy (with or without PFA therapy) has been documented for both VZV and HSV infections (6, 39). Alternative therapies with other antiviral agents that can be tolerated during concurrent zidovudine therapy is an important consideration in the management of severe CMV disease in human immunodeficiency virus-infected individuals.

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