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Activity of 9-(1,3-Dihydroxy-2-Propoxymethyl)Guanine Compared with That of Acyclovir Against Human, Monkey, and Rodent Cytomegaloviruses

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The activities of the purine acyclic nucleoside 9-(1,3-dihydroxy-2-propoxymethyl)guanine (DHPG) against two human and five animal strains of cytomegalovirus were compared with those of acyclovir. DHPG was significantly more active than acyclovir against all but one (mouse cytomegalovirus) of the strains tested, with 50% effective doses ranging from 5 to 13 μM, as determined by plaque reduction assays in human embryonic lung (MRC-5) and human embryonic tonsil cells. Both DHPG and acyclovir inhibited virus replication at concentrations considerably lower than those necessary to inhibit cell proliferation. In mode-of-action studies, the triphosphates of DHPG and acyclovir inhibited human cytomegalovirus DNA polymerase. DHPG phosphorylation to the active triphosphate was enhanced in infected cells; however, this enzymatic activity was unrelated to thymidine kinase. In animal studies, DHPG was slightly more effective than acyclovir in reducing mouse cytomegalovirus-induced mortality.

Much effort has been expended within the last few years to find selective inhibitors of cytomegalovirus (CMV) replication. Although acyclovir is the most active compound in this area, its effectiveness against human CMV appears to be less than against other viruses of the herpes group (27). 2'-Fluoroarabinofuranosyl pyrimidines have shown high activity against human CMV in vitro, but they have a very small therapeutic index (9).

Recent in vitro tests with a novel acyclic nucleoside, 9-(1,3-dihydroxy-2-propoxymethyl)guanine (DHPG, also known as 2'-NDG, BIOLF-62, and BW759U), showed this agent to be significantly more active than acyclovir against strains of human CMV (7, 12, 20, 27, 30, 31, 33). This report deals with the activity of DHPG against a broader range of animal and human CMVs. The effects of DHPG in vivo against mouse CMV also are presented, as are the results of a limited investigation of the mode of action of DHPG.

MATERIALS AND METHODS

Compounds. DHPG, acyclovir, and the triphosphates of each acyclic nucleoside were obtained as described previously (22, 27, 29). The nucleosides used in the thymidine kinase (TK) and DNA polymerase assays were BVdU [(*E*)-5-(2-bromovinyl)-2'-deoxyuridine], provided by Eric DeClercq, Rega Institute of the Catholic University, Leuven, Belgium; FMAU (2'-fluoro-2'-deoxy-5-methyl-arabinofuranosyluracil) and FIAC (2'-fluoro-2'-deoxy-5-iodo-arabinofuranosylcytosine), provided by Jack Fox, Sloan Kettering Institute, New York, N.Y.; and thymidine, 5-iodo-2'-deoxyuridine, 5-bromo-2'-deoxyuridine, 5-trifluorothymidine, dATP, dCTP, and TTP, purchased from Sigma Chemical Co., St. Louis, Mo. Tritiated thymidine (10 Ci/mmol) was from ICN Chemical and Radioisotope Div., ICN Pharmaceuticals Inc., Irvine, Calif. Tritiated DHPG (16 Ci/mmol) and acyclovir (1.6 Ci/mmol) were synthesized by Howard Parnes at Syntex Research, Palo Alto, Calif. Tritiated dGTP (10 Ci/mmol) was from New England Nuclear Corp., Boston, Mass.

Cells and viruses. Human embryonic lung (MRC-5) cells were obtained from M. A. Bioproducts, Walkersville, Md. Human embryonic tonsil (HET; Flow 6000) cells were purchased from Flow Laboratories, Inc., Inglewood, Calif. Squirrel monkey lung (SML) cells were bought from the American Type Culture Collection, Rockville, Md. Primary mouse embryo fibroblast (MEF) cultures were prepared from 17- to 18-day-old Swiss-Webster mouse embryos by trypsinization (25, 28). Primary guinea pig embryo (GPE) cultures were prepared from 30- to 40-day-old embryos of Hartley strain guinea pigs by trypsinization (8, 17). All cells were maintained and passaged in Eagle minimal essential medium containing 10% fetal bovine serum, 10 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) buffer, and 0.12% $NaHCO_3$. No antibiotics were used. The MEF and GPE primary cell cultures were used for assays on passages 2 through 4. The CMVs used were human CMV strains AD169 and Davis, vervet monkey CMV strain CSG, rhesus monkey CMV strain 68-1, squirrel monkey CMV, guinea pig CMV strain 22122, and mouse CMV strain Smith. All viruses were obtained from the American Type Culture Collection. No nonlaboratory-adapted clinical isolates were tested. The human, vervet monkey, and rhesus monkey CMVs were propagated and plaque titers were determined in HET and MRC-5 cells. Squirrel monkey CMV was grown and plaque titers were determined in SML cells. The guinea pig virus was grown in weanling Hartley guinea pigs as described by Griffith and Hsiung (16). Cytopathic effect titration of this virus was performed in GPE cells. Mouse CMV was grown in weanling Swiss-Webster mice as described by Kelsey et al. (18), and plaque titers were determined in MEF cells.

Plaque assays. Confluent monolayers of HET, MRC-5, SML, or MEF cells in 6-well Linbro microplates (Flow Laboratories, Inc., McLean, Va.) were infected with 100 to 200 PFU of virus. After a 1.25-h adsorption period, the virus was aspirated, and Eagle minimal essential medium contain-

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ing 0.5% SeaPlaque agarose (FMC Corp., Marine Colloids Div., Rockland, Maine), 2% fetal bovine serum, 50 μg of gentamicin per ml, and the test compound was applied. Six drug concentrations of each compound were tested in the assay, using three wells per drug dilution and six wells of placebo per test. A second overlay containing drug was added to the human CMV plates on day 4 to maintain cell viability. After 8 days of incubation at 37°C under 5% CO_2, a third overlay containing drug and a final concentration of 0.002% neutral red dye was added to the human CMV plates. For vervet monkey, rhesus monkey, and mouse CMVs, the second overlay contained the drug and the neutral red and was added 3 to 6 days postinfection. Plaques were counted 4 to 24 h after applying the neutral red overlay. Squirrel monkey CMV plaques stained very poorly with neutral red; therefore, the plates were processed by fixing cells in 10% buffered Formalin for 15 min, removing the agarose overlay, and then staining the cells with 10% Gram crystal violet (Difco Laboratories, Detroit, Mich.). All plaques were counted at ×17 with a Bellco plaque viewer. Drug concentrations which reduced plaque numbers by 50% (50% effective dose) were calculated with a computer with a semilog probit analysis program (13).

Virus yield reduction assays. To determine antiviral activity against guinea pig CMV, 2×10^5 GPE cells per well were seeded in Falcon (Becton Dickinson Labware, Oxnard, Calif.) 24-well plates. The medium was aspirated 4 h later, and replaced with medium containing 10^4 50% tissue culture infective doses of guinea pig CMV per ml and drug at various concentrations. Maximal cytopathic effect was observed in drug-free cultures after 7 to 10 days of incubation. The medium and cells in each well were sonicated and stored at −80°C until assayed for virus yield. These titrations were conducted in 96-well plates (Corning Glass Works, Corning, N.Y.) by an endpoint dilution method (26). Four wells were used per $\log_{10}$ dilution of virus-containing medium. The medium was changed at 24 h to remove residual drug carried over from the first part of the experiment. The dose of compound which caused a 1-log reduction in virus yield was determined as the endpoint in this assay.

Cell cytotoxicity assays. The effects of DHPG and acyclovir on cell proliferation were determined in Linbro 6-well plates seeded with 3×10^4 MRC-5, MEF, or SML cells or with 1×10^4 HET or GPE cells per well. After 24 h, Eagle minimal essential medium containing 10% serum and drug at various concentrations was added to the wells. Three wells were used per drug dilution, and for the control six wells were used per assay. After approximately five cell doublings, drug was removed and cell numbers were determined. Drug concentrations which reduced cell numbers by 50% (50% inhibitory dose) were calculated by probit analysis.

DNA polymerase purification and assay. A 5-ml pellet of human CMV-infected MRC-5 cells was sonicated in 50 mM K_2HPO_4 (pH 8.0) buffer and then centrifuged at 30,000 × *g* for 30 min. The supernatant was batch absorbed to 5 g of DE-52 (Whatman, Inc., Clifton, N.J.) for 30 min at 4°C. After washing the DE-52 with buffer, the enzyme was eluted with a buffer (8 ml) containing 200 mM K_2HPO_4 (pH 8.0), 20% glycerol, 0.2 mM phenylmethylsulfonyl fluoride (a protease inhibitor), and 2 mM dithiothreitol. The same procedure was used to obtain the α DNA polymerase from uninfected cells. The cell β polymerase was not present in the preparation since it does not adhere to DE-52. Further purification of the viral polymerase by other chromatographic techniques resulted in its inactivation. Confirmation of viral enzyme activity in preparations obtained from infected cells was made by assaying in the presence of 150 mM KCl. Under these conditions the cellular enzyme is inactive. The 100-μl assay mixture for kinetic analyses contained 50 mM Tris hydrochloride (pH 8.0), 8 mM $MgCl_2$, 0.5 mg of bovine serum albumin per ml, 100 μM dATP, 100 μM dCTP, and 100 μM TTP. KCl at 150 mM was present for viral enzyme assays. [^{3}H]dGTP was used to compete against DHPG triphosphate (DHPGTP) and acyclovir triphosphate (ACVTP). Samples were incubated at 37°C for 30 min and then spotted on Whatman GF/C glass filter disks. Filters were washed three times in 5% trichloroacetic acid–10 mM PP_i and once in methanol. Dry filters were counted for radioactivity in scintillation vials. K_m and K_i values were estimated from Lineweaver-Burk plots.

Preparation of cell extracts for nucleotide analysis. MRC-5 cells in T-25 flasks were left uninfected or were infected with human CMV (strain AD169) at a multiplicity of infection of 2 PFU per cell. After virus adsorption, all flasks received 10 μM [^{3}H]DHPG. Each day, the cells from a group of flasks were pelleted after trypsinization, and the dry pellets were stored at −80°C before analysis. Nucleotide extracts were prepared by breaking up the pellets in 3.5% perchloric acid. After 30 min at 4°C, neutralization of samples was done with 1 N KOH containing 0.2 M imidazole (1,3-diazo-2,4-cyclopentadiene). A 425-μl portion of each sample (equivalent to extract from 2×10^6 cells) was assayed as detailed below.

High-pressure liquid chromatographic analysis of cell extracts. DHPG nucleotides extracted from cells were analyzed with a high-pressure liquid chromatographic apparatus fitted with a Whatman SAX 10/25 Partisil column. A linear gradient of 0.01 M to 1 M KH_2PO_4 (pH 3.5) was run for 35 min, followed by maintenance of the 1 M buffer for an additional 15 min to elute all of the triphosphate. Under these conditions, the mono-, di-, and triphosphate peaks of DHPG eluted at 11, 18, and 31 min, respectively. Fractions containing [^{3}H]DHPG were collected at 1-min intervals with a fraction collector fitted with a scintillation vial rack. Aquasol (New England Nuclear Corp.) was used as scintillation fluid. The amount of nucleotides were quantified as previously described (29).

TK purification and kinetic analysis. Cytosol and mitochondrial TKs were purified from MRC-5 cells and SML cells by a modification of the Lee and Cheng procedure (19). A linear gradient of 100 mM Tris hydrochloride (pH 7.5) to 1 M Tris hydrochloride (pH 7.5)–200 μM thymidine was run to elute cytosol TK. A buffer containing 1.5 M Tris hydrochloride (pH 7.5)–300 μM thymidine eluted mitochondrial TK. Both buffers also contained 1.5 mM ATP–Mg^{+2}, 10% glycerol, 1 mg of bovine serum albumin per ml, and 3 mM dithiothreitol. The two cellular isozymes could be distinguished by a competitive assay in which BVdU inhibited [^{3}H]thymidine phosphorylation of mitochondrial TK but did not inhibit cytosol TK (5). The procedure described above also was used to purify a TK activity induced in squirrel monkey CMV-infected SML cells. Enzyme reaction mixtures (100 μl) contained 100 mM Tris hydrochloride (pH 7.5), 50 mM KCl, 2 mM $MgCl_2$, 2 mM ATP, 25 mM NaF, 1 mg of bovine serum albumin per ml, ^{3}H-nucleoside, and enzyme. Determination of K_m values was performed by running various concentrations of [^{3}H]thymidine and enzyme for 1 h. In analyzing SML cytosol and squirrel monkey CMV-induced TK activities, competitive studies were run with [^{3}H]thymidine and various compounds such as iododeoxyuridine, bromodeoxyuridine, trifluorothymidine, FIAC, and FMAU; the first three compounds were run at 0.2

TABLE 1. Antiviral and anticellular activities of DHPG and acyclovir

CMV[a]	Plaque inhibition ED_{50}[b] (μM)		Cell type	Cell proliferation ID_{50}[c] (μM)		Therapeutic index	
	DHPG	Acyclovir		DHPG	Acyclovir	DHPG	Acyclovir
Human (AD169)	7 ± 3	95 ± 10	MRC-5	110 ± 50	1,575 ± 140	16	17
	7 ± 2	55 ± 10	HET	250 ± 80	1,360 ± 260	36	25
Human (Davis)	7 ± 1	64 ± 4	MRC-5	110 ± 50	1,575 ± 140	16	25
	5 ± 2	39 ± 10	HET	250 ± 80	1,360 ± 260	50	35
Vervet monkey (CSG)	13 ± 4	51 ± 1	MRC-5	110 ± 50	1,575 ± 140	8	31
	9 ± 5	40 ± 22	HET	250 ± 80	1,360 ± 260	28	34
Rhesus monkey (68-1)	8 ± 2	39 ± 14	MRC-5	110 ± 50	1,575 ± 140	14	40
	7 ± 2	40 ± 15	HET	250 ± 80	1,360 ± 260	36	34
Squirrel monkey	1.3 ± 1	28 ± 19	SML	1,500 ± 950	1,820 ± 980	1,150	65
Guinea pig (22122)	70 ± 30	700 ± 230	GPE	2,900 ± 84	1,200 ± 650	41	2
Mouse (Smith)	11 ± 1	1 ± 0.8	SML	210 ± 80	180 ± 70	19	180

[a] Virus strains are within parentheses.
[b] The 50% effective dose (ED_{50}) ± standard deviation was determined by three independent plaque assays for all viruses except guinea pig CMV. The endpoint for the guinea pig virus was a 1-log reduction in virus yield.
[c] The 50% inhibitory dose (ID_{50}) ± standard deviation was determined by cell proliferation assays for all viruses.

to 10 μM against thymidine (1 μM), and the last two were run at 100 to 900 μM against thymidine. The DE-81 paper method (15) was used to assay each reaction.

Animal studies. Weanling Swiss-Webster female mice (Charles River Breeding Laboratories, Wilmington, Mass.) weighing approximately 11 g each were infected intraperitoneally with mouse CMV. The mice received 3.2×10^4 PFU for an acute lethal infection. DHPG and acyclovir were administered subcutaneously twice daily (at 9 a.m. and 3 p.m.) for 5 days, starting 6 h postinfection. In each experiment, there were initially 20 mice per dosage group, including the saline control group.

RESULTS

Antiviral and anticellular activity in vitro. Against human, monkey, and guinea pig CMVs, DHPG was much more active than acyclovir (Table 1). Only against mouse CMV was the activity of acyclovir greater than that of DHPG. There did not appear to be much difference in 50% effective dose values obtained for each drug when the MRC-5 or HET cell line was used.

Cell proliferation studies were conducted in uninfected cells to determine drug-induced cytotoxicity by DHPG and acyclovir (Table 1). DHPG inhibited cell proliferation at 5- to 15-fold-lower concentrations in MRC-5 and HET cells compared with proliferation with acyclovir. Fifty percent infective doses of both compounds in SML and MEF cells were nearly equivalent, whereas acyclovir was slightly more toxic than DHPG in GPE cells.

Animal studies. Various doses of compound were tested for efficacy against mice infected with CMV (Table 2, experiment 1). DHPG and acyclovir caused statistically significant increases in numbers of survivors at doses of ≥10 and ≥25 mg/kg, respectively. Increases in mean survival times were noted at lower drug doses. In a second experiment, in which treatments began either 6, 24, 48, 72, or 96 h postinoculation with a single dose of drug (Table 2, experiment 2), DHPG was significantly active at reducing mortality when therapy was started at 48 h, whereas acyclovir was protective only when treatments were initiated at 24 h or earlier. Absence of a clear dose response was seen in repeated tests with acyclovir.

Mode-of-action studies. DNA polymerases partially purified from MRC-5 infected and uninfected cells were evaluated for inhibition by DHPGTP and ACVTP. In these assays, ACVTP was approximately sixfold more active than DHPGTP at inhibiting the viral enzyme (K_i values of 0.3 μM for ACVTP and 1.7 μM for DHPGTP). DHPGTP showed a greater degree of inhibition of the viral DNA polymerase than the cellular α enzyme (K_i values of 1.7 and 17 μM, respectively). ACVTP was not evaluated against the cellular polymerase.

Since DHPGTP was found to be the active moiety inhibiting the virus DNA polymerase, we decided to examine the enzymatic phosphorylation of DHPG in virus-infected and uninfected cells. Amounts of DHPG nucleotides were monitored in cells over 4 days (Table 3). On each day the amount of DHPGTP was at least 10-fold higher than the amount present in uninfected cells.

The enzyme or enzymes responsible for conversion of DHPG to the monophosphate were then investigated. In dialyzed crude enzyme preparations, DHPG kinase activity was increased by about eightfold when compared with the activity in uninfected cell extracts (Table 4). In contrast, acyclovir kinase was stimulated only twofold in infected cells. The phosphorylation of the natural nucleosides was also increased in infected cells, which shed no light on a particular kinase activity being responsible for DHPG phosphorylation.

Since DHPG is phosphorylated by herpes simplex virus TKs (12, 30), cellular TKs have been implicated as target enzymes for DHPG phosphorylation in human CMV-infected cells. For this reason, we examined TKs from various sources as the putative DHPG kinase. In crude extracts of virus-infected cells, the levels of TK activity induced by vervet monkey, rhesus monkey, guinea pig, and human CMVs were no greater than threefold more than that of uninfected control cultures. However, in cultures infected with squirrel monkey CMV, a 40-fold increase in TK activity was observed with little DHPG kinase activity present.

TABLE 2. Effects of DHPG and acyclovir on mouse CMV (Smith strain)-induced mortality in mice

Drug (mg/kg)[a]	Time (h) of first treatment[b]	No. of survivors/ total no. (% survival)	Mean survival time ± SD (days)[c]
Expt 1			
Saline	6	2/20 (10)	4.4 ± 0.78
DHPG			
1	6	2/20 (10)	6.2 ± 1.8[d]
5	6	2/20 (10)	6.3 ± 1.4[d]
10	6	8/20 (40)[e]	7.7 ± 1.8[d]
25	6	15/20 (75)[e]	6.4 ± 0.55[d]
50	6	19/20 (95)[e]	7.0 ± 0.0[d]
Acyclovir			
1	6	1/20 (5)	4.8 ± 0.79
5	6	2/20 (10)	5.7 ± 1.0[d]
10	6	1/20 (5)	5.9 ± 1.0[d]
25	6	15/20 (75)[e]	6.8 ± 2.5[d]
50	6	7/20 (35)	7.6 ± 1.7[d]
Expt 2			
Saline	6	2/19 (11)	5.2 ± 1.2
DHPG			
50	6	18/20 (90)[e]	6.5 ± 0.71
50	24	15/19 (79)[e]	10.7 ± 3.8[d]
50	48	9/19 (47)[e]	8.0 ± 2.5[d]
50	72	6/20 (30)	6.1 ± 1.6
50	96	1/20 (5)	4.8 ± 0.71
Acyclovir			
50	6	10/19 (53)[e]	7.0 ± 1.2[d]
50	24	12/20 (60)[e]	7.7 ± 1.9[d]
50	48	0/20 (0)	8.2 ± 2.2[d]
50	72	7/20 (35)	5.8 ± 2.9
50	96	4/19 (21)	4.3 ± 0.62

[a] Half-daily doses were administered at 9 a.m. and 3 p.m. for 5 days.
[b] Time of first treatment after virus inoculation.
[c] Mean survival time of the mice that died.
[d] Statistically significant ($P < 0.05$) by Fisher exact test.
[e] Statistically significant ($P < 0.05$) by Mann-Whitney U-test.

Upon purifying the TK from squirrel monkey CMV-infected cells, it was found that the retention time on the affinity column was similar to that of cytosol TK (the cytosol and CMV-induced TKs eluted at 0.3 M Tris–50 mM thymidine to 0.5 M Tris–100 mM thymidine). In addition, each enzyme showed a similar pattern of inhibition by compounds such as trifluorothymidine, iododeoxyuridine, and bromodeoxyuridine and a lack of inhibition by FIAC and FMAU. We concluded from this that the squirrel monkey CMV induced high levels of cytosol TK rather than specifying a unique enzyme. Affinity-purified MRC-5 and SML cell cytosol and mitochondrial TKs and squirrel monkey CMV-induced cytosol TK were analyzed for thymidine, DHPG, and acyclovir phosphorylating activities. The cytosol enzymes had K_m values for thymidine of 0.51 to 0.59 μM. K_m values for the mitochondrial enzymes were 0.32 to 0.38 μM. DHPG and acyclovir were not phosphorylated to any degree at concentrations of ≤100 μM. In addition, neither DHPG nor acyclovir at 300 μM was inhibitory to the phosphorylation of 1 μM thymidine.

We also tried to purify and characterize DHPG kinase activity directly from 5-ml pellets of human CMV-infected MRC-5 cells with [^{3}H]DHPG (200 μM) and 2 mM ATP as substrates to assay fractions from various chromatography columns. In repeated attempts, not enough activity was ever recovered to characterize the enzyme.

TABLE 3. DHPG metabolism in human CMV (AD169)-infected and uninfected MRC-5 cells

Day post-inoculation	DHPG nucleotides (pmol/10^6 cells)[a]					
	Monophosphate		Diphosphate		Triphosphate	
	Uninfected	Infected	Uninfected	Infected	Uninfected	Infected
1	0.03	0.17	0.07	0.65	0.52	5.7
2	0.14	0.09	0.10	0.57	0.41	5.5
3	0.07	0.84	0.15	2.5	0.08	7.3
4	0.01	0.05	0.05	0.29	0.29	2.4

[a] [^{3}H]DHPG (10 μM) at 50 μCi/ml was required to detect quantifiable peaks of DHPG nucleotides by high-pressure liquid chromatography. The extracted nucleotides from 2 × 10^6 cells were analyzed per injection.

DISCUSSION

These studies showed DHPG to be an effective inhibitor of several types of CMV, most importantly human CMV. This is in contrast to the lower degree of activity of acyclovir, which showed exceptional potency only against mouse CMV. Others have reported significant activity of DHPG against other strains of human CMV (7, 12, 33).

There is some correlation between the cytotoxicity of DHPG and acyclovir in uninfected cells and their antiviral potencies in the MRC-5, HET, and MEF cell lines. In those lines in which DHPG was more cell inhibitory, its activity against the virus was superior to that of acyclovir. In mouse cells, in which acyclovir was slightly more cytotoxic than DHPG, the former compound also was more effective against virus replication in vitro. Presumably, higher toxicity in these cell lines may be correlated with higher levels of intracellular nucleoside triphosphate. In a previous report, Tyms and colleagues concluded that the greater activity of acyclovir against mouse CMV than against human CMV was related to differing sensitivities of human versus mouse cells (34). In GPE cells, in which neither compound was particularly inhibitory to cell proliferation, low antiviral potency also was observed. In contrast to the above are the comparative results of drug effect against SML cells and squirrel

TABLE 4. Stimulation of nucleoside kinase activities in human CMV (AD169)-infected cells

Substrate for kinase[a]	Stimulation of activity (pmol/min per ml of enzyme)		Ratio of infected/ uninfected
	Uninfected	Infected	
2′-Deoxyadenosine	4.2	136.0	32.0
2′-Deoxycytidine	18.0	49.0	2.7
2′-Deoxyguanosine	2.7	59.0	22.0
Thymidine	16.0	28.0	1.8
DHPG	0.41	3.2	7.8
Acyclovir	0.52	1.2	2.3
Adenosine	1,300.0	5,670.0	4.4
Cytidine	30.0	141.0	4.7
Guanosine	5.1	24.0	4.7
Uridine	7.5	113.0	15.0

[a] The enzyme sources used for all reactions were dialyzed crude extracts of infected or uninfected MRC-5 cells. Each nucleoside was at 200 μM in the reaction mixture.

monkey CMV. Low cytotoxicity was observed and good antiviral activity was achieved in this system with DHPG and acyclovir, although the selectivity of acyclovir was less than that achieved against mouse CMV.

For DHPG and acyclovir to be less cytotoxic and more virus inhibitory against certain virus-cell systems suggests a greater degree of inhibition of viral processes over host functions. This report showed that in two respects such selectivity exists with DHPG in human CMV-infected cells. First, more DHPGTP is produced in infected than uninfected cells, and second, DHPGTP is a more potent inhibitor of viral than of cellular DNA polymerase. Other investigators have shown that viral DNA synthesis and some early viral polypeptides are inhibited in human CMV-infected cells (20, 33). However, herpes viral polypeptides are not inhibited by DHPG (6). Biron et al. showed a 10-fold increase in the production of DHPGTP over ACVTP in human CMV-infected cells and a 100-fold increase of DHPGTP over DHPGTP in uninfected cells (1). Presumably, the higher input multiplicity of virus used in our studies relative to the previous investigation (1) was detrimental to enzyme induction and subsequent DHPGTP production.

It was not surprising that ACVTP was a more potent inhibitor of the human CMV DNA polymerase than was DHPGTP. The same effect was demonstrated for these compounds against the herpes simplex virus DNA polymerase (32). Our results agree closely with those obtained by Biron et al. (2); however, Mar and colleagues found DHPGTP and ACVTP to be more active against the DNA polymerase of the Towne strain of human CMV (21). The question that remains is whether DHPG may have an additional action against some other aspect of viral DNA synthesis that would account for it ultimately being more potent than acyclovir as a CMV inhibitor. The increase in intracellular levels of DHPGTP may provide some explanation but probably is not the full answer to its potency relative to acyclovir.

In this investigation, we ruled out the possibilities that CMV-induced TK or cytosol and mitochondrial TKs are involved in the phosphorylation of DHPG and acyclovir in vitro. Datta and Pagano (A. K. Datta and J. S. Pagano, Program Abstr. 23rd Intersci. Conf. Antimicrob. Agents Chemother, abstr. no. 918, 1983) also have shown this with mouse CMV. Acyclovir has been shown not to be a substrate for human leukemia cell cytosol and mitochondrial TKs (5). The exact enzyme or enzymes that phosphorylate these compounds in uninfected or CMV-infected cells are at present unknown. Datta and Pagano provide evidence that in a Burkitt lymphoma cell line, the enzyme responsible for acyclovir phosphorylation may be cytosol deoxycytidine kinase (10). Others suggest that the enzyme is a cellular deoxyguanosine kinase (23, 24). The increase in DHPGTP in human CMV-infected cells is not the same as that observed with acyclovir and FIAC (9, 14). The latter two compounds are phosphorylated to relatively the same degree in infected and uninfected cells. This stimulated phosphorylation of DHPG in infected cells does not necessarily imply the existence of a virus-specified enzyme. More likely DHPG kinase is an induced cellular enzyme.

It is interesting that DHPG exhibited enhanced efficacy over acyclovir in mice infected with CMV, although showing weaker in vitro activity against the virus. Against herpes simplex viruses, the two drugs show similar in vitro effects, yet DHPG is markedly more potent in vivo (12, 30). These data suggest that the pharmacology of DHPG may be favorable in increasing its antiviral activity. It recently was reported that DHPGTP persists longer than ACVTP in herpes-infected cells after drug removal from infected cultures (29), which would give DHPG an advantage in an in vivo situation where blood levels of drug are dynamic. DHPGTP also was found to persist in human CMV-infected cells (1). Preliminary pharmacokinetic studies (unpublished data) show that the metabolism of DHPG is similar to acyclovir in monkeys, dogs, and mice, which indicates that intracellular rather than extracellular pharmacokinetics differentiate the two nucleotides.

The data on the lack of virus-specified TK activities expands the list of CMVs that do not encode a unique enzyme. The squirrel monkey CMV is similar to human CMV in its ability to induce greater levels of cytosol TK (11). Although mouse CMV was not tested here, reports in the literature indicate that the Smith strain of virus cannot induce TK activity (3, 4).

In summary, this investigation shows that the acyclic nucleoside DHPG is significantly inhibitory against several strains of CMV. It also is nontoxic to uninfected cells at antiviral concentrations and is effective in vivo in a mouse CMV model. Its mode of action involves inhibition of the viral DNA polymerase and selective phosphorylation in virus-infected cells.

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