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The N-7-Substituted Acyclic Nucleoside Analog 2-Amino-7-[(1,3-Dihydroxy-2-Propoxy)Methyl]Purine Is a Potent and Selective Inhibitor of Herpesvirus Replication

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2-Amino-7-[(1,3-dihydroxy-2-propoxy)methyl]purine (compound S2242) represents the first antivirally active nucleoside analog with the side chain attached to the N-7 position of the purine ring. Compound S2242 strongly inhibits the in vitro replication of both herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) (50% effective concentration [EC_{50}], 0.1 to 0.2 μg/ml), varicella-zoster virus (EC_{50}, 0.01 to 0.02 μg/ml) and thymidine kinase (TK)-deficient strains of HSV (EC_{50}, 0.4 μg/ml) and varicella-zoster virus (EC_{50}, 0.2 to 0.5 μg/ml). Potent activity was also observed against murine cytomegalovirus (EC_{50}, 1 μg/ml), human cytomegalovirus (HCMV) (EC_{50}, 0.04 to 0.1 μg/ml), and human herpesvirus 6 (EC_{50}, 0.0005 μg/ml). Compound S2242 (i) was not cytotoxic to confluent Vero, HeLa, or human fibroblast cells at concentrations of >100 μg/ml, (ii) proved somewhat more cytostatic to Vero, HEL, HeLa, and C127I cells than ganciclovir, and (iii) was markedly more cytostatic than ganciclovir to the growth of the human lymphocytic cell lines HSB-2 and CEM°. In contrast to ganciclovir, (i) compound S2242 proved not to be cytocidal to murine mammary carcinoma (FM3A) cells transfected with the HSV-1 or HSV-2 TK gene, (ii) exogenously added thymidine had only a limited effect on its anti-HSV-1 activity, and (iii) the compound was not phosphorylated by HSV-1-encoded TK derived from HSV-1 TK-transfected FM3A cells, indicating that the compound is not activated by a virally encoded TK. Compound S2242 inhibited (i) the expression of late HCMV antigens at an EC_{50} of 0.07 μg/ml (0.6 μg/ml for ganciclovir) and (ii) HCMV DNA synthesis at an EC_{50} of 0.1 μg/ml (0.32 μg/ml for ganciclovir), i.e., values that are close to the EC_{50}s for inhibition of HCMV-induced cytopathogenicity. Neither ganciclovir nor S2242 had any effect on the expression of immediate-early HCMV antigens, which occurs before viral DNA synthesis. In time-of-addition experiments, S2242 behaved like ganciclovir and acyclovir; i.e., the addition of the drugs could be delayed until the onset of viral DNA synthesis.

Agents approved in one or more countries to treat herpesvirus infection include idoxuridine, trifluridine, vidarabine, and acyclovir for the topical treatment of herpetic eye infection; vidarabine and acyclovir for the systemic treatment of herpes encephalitis, acyclovir for the topical and systemic (oral or intravenous) treatment of genital herpes; acyclovir for the systemic (intravenous, oral) treatment of herpes simplex virus (HSV) or varicella-zoster virus (VZV) infections in immunosuppressed patients; and ganciclovir and foscarnet for the systemic (intravenous) treatment of human cytomegalovirus (HCMV) retinitis in patients with AIDS. Foscarnet is effective in the treatment of infections with acyclovir-resistant thymidine kinase-deficient (TK^-) HSV or VZV mutants and ganciclovir-resistant HCMV infections (5, 16, 18, 25). However, double resistant viruses (i.e., ganciclovir- and foscarnet-resistant HCMV and acyclovir- and foscarnet-resistant HSV) have been reported previously (8, 13).

Jähne et al. (11) reported the synthesis of a series of N-7-substituted acyclic nucleoside analogs. In this report we describe the antiviral activity of 2-amino-7-[(1,3-dihydroxy-2-propoxy)methyl]purine (compound S2242). This compound appears to be a strong inhibitor of the replication of herpesviruses, particularly CMV and TK^- strains of HSV and VZV. In attempts to determine the mode of antiviral action of compound S2242, we investigated its inhibitory effect on the replicative cycle of herpesviruses, in particular HCMV.

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MATERIALS AND METHODS

Compounds. The synthesis of compound S2242 has been reported previously (11). The structural formula of the compound is depicted in Fig. 1. Acyclovir (ACV; Zovirax) was obtained from Wellcome Research Laboratories (Aalst, Belgium), ganciclovir (DHPG; Cytovene) was obtained from Sarva-Syntex (Brussels, Belgium), and foscarnet (Foscavir) was obtained from Astra Pharmaceutical Products, Inc. (Södertalje, Sweden). (*E*)-5-(2-Bromovinyl)-2′-deoxyuridine (BVDU) was synthesized as described previously (12), and (*S*)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine (HPMPC) was kindly provided by N. Bischofsberger (Gilead Sciences, Foster City, Calif.).

Viruses. The following virus strains were used: HSV type 1 (HSV-1; strain KOS) and type 2 (HSV-2; strain G), TK^- HSV-1 (B2006) (6), HCMV Davis (ATCC VR-807) and AD-169 (ATCC VR-538), murine CMV (MCMV) Smith (ATCC VR-194), VZV OKA (ATCC VR-795) and YS and VZV TK^- strains 07/1 and YS/R; human herpes virus 6 was a clinical isolate kindly provided by K. Schneweis (University of Bonn, Bonn, Germany); vaccinia virus, coxsackievirus B4 and poliovirus type 1, parainfluenza virus type 3 (ATCC VR-93), reovirus type 1 (ATCC VR-230), Sindbis virus and Semliki

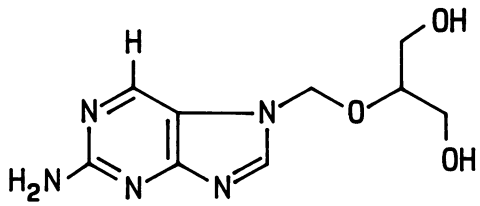

FIG. 1. Structural formula of 2-amino-7-[(1,3-dihydroxy-2-propoxy)methyl]purine (compound S2242).

forest virus (ATCC VR-67), influenza A virus Ishikawa/222/82 (H3N2) and influenza B virus Singapore/222/79, respiratory syncytial virus Long, arenaviruses Junin and Tacaribe, and human immunodeficiency virus type 1 (HIV-1; HTLV-IIB) and type 2 (HIV-2; LAV-2ROD) (6, 7, 10, 22).

Antiviral assays. For all viruses (except HCMV, VZV, human herpesvirus 6, influenza virus, and HIV-1 and -2) confluent cultures of human embryonic skin muscle (E_6SM), HeLa, or Vero cells in microtiter trays were inoculated with virus at 100 times the 50% cell culture infective dose per well. After a 2-h incubation period, virus was removed and the test compounds were added to the cultures at various concentrations. Virus-induced cytopathogenicity was recorded at 1 to 2 days postinfection for VSV, at 2 days for coxsackievirus, Semliki forest virus, and poliovirus, at 2 to 3 days for HSV-1, HSV-2, TK^- HSV-1, vaccinia virus and Sindbis virus, and at 5 days for reovirus and arenaviruses. For the anti-HCMV and anti-VZV assays human embryonic lung (HEL) fibroblasts were infected with 100 PFU of HCMV or 20 PFU of VZV per well, and for the anti-MCMV assays C127I cells in microtiter trays were infected with 20 PFU of MCMV per well. Compounds were added after a 2-h incubation period, and the cells were further incubated at 37°C. Plaques (VZV and MCMV) or virus-induced cytopathogenicity (HCMV) was recorded after 5 days (VZV, MCMV) and 7 days (HCMV), respectively, as described previously (1, 28). The anti-HHV-6 activity was evaluated on JJHAN cells (a permanent human B-lymphocyte cell line kindly provided by K. Schneweis). JJHAN cells were cultivated in RPMI 1640 medium supplemented with 20% fetal calf serum (FCS). A total of 4×10^5 cells in 2 ml of medium were infected by the addition of 0.25 ml of a supernatant cell culture infected for 11 days. Microscopic examination of syncytium formation was performed 10 to 11 days postinfection. Antimyxovirus activity was assessed on MDCK (influenza virus type A and B) or HeLa (respiratory syncytial virus) cells. Cells were infected with 20 50% cell culture infective doses of virus, and after a 2 h adsorption period the appropriate concentrations of compounds were added, after which the cultures were incubated for an additional 5 days at 35°C. Evaluation of the anti-HIV activity of the compounds was assessed by the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] method as described previously (21).

Cytotoxicity and cytostatic assay. HEL cells were seeded at a ratio of 4.5×10^3 cells per well of 96-well microtiter plates in Eagle's minimal essential medium containing 20% FCS. Appropriate concentrations of the test compounds were added in medium supplemented with 2% FCS, and the cells were allowed to proliferate for 4 days. Then, the cells were detached by trypsinization and counted with a Coulter counter. The cytotoxicities for Vero and C127I cells were assessed similarly. Alternatively, cell proliferation was followed over a period of 4 to 8 days (see Fig. 2 and 3). The 50% cytostatic concentration was estimated from graphic plots. Human (CEM° and HSB-2) and murine (L1210) cell lines were seeded in microtiter plates at 5×10^4 cells per well in the absence or presence of compounds. The cells were counted with a Coulter counter after they were allowed to proliferate for 48 h. FM3A cells (subclone F287) were originally established from a spontaneous mammary carcinoma in a C3H/He mouse (2) and were designated FM3A/0. The FM3A TK^-/HSV-1 TK^+ and FM3A TK^-/HSV-2 TK^+ cell lines, which lack host cell TK activity but contain either the HSV-1 TK or the HSV-2 TK gene, were derived from the FM3A/TK^- cells as described previously (3, 24). Cytostatic effects were determined as described above for CEM, HSB-2, and L1210 cells. The cytotoxicities of the compounds was determined on confluent cultures of Vero, HeLa, and MRC-5 cells after a 20-h incubation with the test compounds at 37°C by the XTT (2,3-bis[2-methoxy-4-nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxanilide inner salt) method according to the manufacturer's instruction (Sigma Chemical Company). Determination of [*methyl*-^{3}H]thymidine ([*methyl*-^{3}H]dThd; specific radioactivity, 46 Ci/mmol), [^{3}H]uridine ([^{3}H]Urd; specific radioactivity 21 Ci/mmol), and L-[*methyl*-^{3}H]methionine (specific activity, 71 Ci/mmol) incorporation in growing HEL and Vero cells was performed as described previously (19).

TK assay. HSV-1-encoded TK was derived from FM3A TK^-/HSV-1 TK^+ cells after partial purification over DEAE-Sepharose as described previously (4).

Analysis of HCMV-induced antigens in HEL cell cultures. Confluent HEL cell monolayers in two-well tissue culture chamber slides (Nunc, Naperville, Ill.) were infected with HCMV Davis at a multiplicity of infection of approximately 0.2. A monoclonal antibody directed against an immediate-early antigen (IEA monoclonal antibody E13; Biosoft, Paris, France) or against a late antigen (monoclonal antibody 10/8; kindly provided by S. Michelson, Institute Pasteur, Paris, France) was used to monitor HCMV antigen expression at 1 or 7 days postinfection, respectively. Analysis of antigen expression was performed by a fluorescence microscopic assay as described before (23). Quantitation of IEA expression by immunofluorescence microscopy was done by counting the number of IEA-positive cells in 10 to 20 microscopic fields.

Analysis of HCMV DNA synthesis in HEL cells. Confluent HEL cell cultures in 60-mm petri dishes were infected with HCMV Davis at a multiplicity of infection of approximately 0.4. After a 2-h adsorption period the appropriate concentrations of compounds were added. The cell cultures were incubated for 4 days, and 17 μCi of [*methyl*-^{3}H]dThd was added to the medium 24 h before harvesting the cells. The cell monolayers were washed once with phosphate-buffered saline, and the cells were lysed with 200 μl of a solution containing 0.2% sodium dodecyl sulfate, 0.5% *N*-laurylsarcosylate, 1 mM sodium EDTA, 100 mM NaCl, and 10 mM Tris · HCl (pH 7.4). Each sample (200 μl) was layered on top of 8 ml of a CsCl solution (density, 1.7067 g/ml) and was centrifuged to equilibrium at 30,000 rpm in a Beckman L7-55 ultracentrifuge for 65 h at 20°C. Seven-drop fractions were collected from the bottoms of the tubes, the refractive index of every fifth fraction was determined, and the acid-insoluble material of the fractions was precipitated on Whatman GF/C filters (Whatman International, Maidstone, United Kingdom) with 5% ice-cold trichloroacetic acid. Filters were dried with ethanol, and the radioactivity was determined in a toluene-based scintillant.

TABLE 1. Anti-DNA virus activity of compound S2242 and some selected compounds

Virus	EC_{50} (μg/ml)[a]				
	S2242	DHPG	BVDU	HPMPC	ACV
HSV-1 KOS	0.26 ± 0.09	0.0070 ± 0.0005	0.04 ± 0.02	0.48 ± 0.15	0.02 ± 0.01
HSV-1 B2006 (TK^-)	0.4 ± 0.2	20	>400	0.47 ± 0.32	37 ± 23
HSV-2 G	0.13 ± 0.06	0.004 ± 0.002	200	0.60 ± 0.4	0.03 ± 0.1
VZV OKA	0.010 ± 0.008	0.03 ± 0.01	0.0008 ± 0.0002	0.08 ± 0.02	0.2 ± 0.13
VZV YS	0.02 ± 0.002	0.02 ± 0.002	0.0015 ± 0.001	0.14 ± 0.06	0.4 ± 0.3
VZV 07/1 (TK^-)	0.05 ± 0.04	0.1 ± 0.0	30 ± 20	0.07 ± 0.03	14 ± 5.1
VZV YS/R (TK^-)	0.02 ± 0.02	0.3 ± 0.1	38 ± 32	0.02 ± 0.02	16 ± 5.3
HCMV AD169	0.12 ± 0.2	0.6 ± 0.3	>100	0.03 ± 0.02	
HCMV Davis	0.04 ± 0.01	0.7 ± 0.7	>100	0.04 ± 0.02	23 ± 3
MCMV Smith	1.0 ± 0.1	5.0 ± 0.05		0.10 ± 0.02	
Vaccinia virus	0.4 ± 0.3		0.3 ± 0.1	3.0 ± 1.0	

[a] Data are means ± standard deviations for two to five separate experiments. EC_{50}, 50% effective concentration, or the concentration required to reduce virus-induced cytopathicity or plaque formation by 50%.

RESULTS

Antiviral spectrum of compound S2242 in vitro. The antiviral activity of compound S2242 was evaluated against a wide array of viruses (Table 1). The compound proved to be markedly active against HSV-1 and HSV-2, although it was one to two orders of magnitude less active than acyclovir and ganciclovir. In contrast to ACV, DHPG, and BVDU, which depend for their activation on the HSV-induced TK, compound S2242 afforded marked activity against TK-deficient strains of HSV-1. Also, compound S2242 showed equipotent activity against TK^+ and TK^- strains of VZV. The activity of S2242 against HCMV was 5- to 20-fold more pronounced than that of ganciclovir, and the compound proved to be 5-fold more active than ganciclovir against MCMV replication. Potent activity was also observed against vaccinia virus (poxvirus) replication and human herpesvirus 6 (50% inhibitory concentration, 0.0005 μg/ml, compared with 22 μg/ml for DHPG). No activity of the compound was observed against orthomyxovirus (influenza A and B viruses), paramyxovirus (respiratory syncytial virus), arenavirus Junin and Tacaribe, picornaviruses (coxsackievirus B4, poliovirus), rhabdoviruses (vesicular stomatitis virus), parainfluenza virus, reovirus, togaviruses (Sindbis virus and Semliki forest virus), and retroviruses (HIV-1 and HIV-2).

Compound S2242 appeared to be somewhat more cytostatic than ganciclovir for uninfected HEL, Vero, HeLa, and C127I cells (50% cytostatic concentrations, 10 to 30 μg/ml) (Table 2 and Fig. 2 and 3) but did not alter the normal cell morphologies of confluent cells at a concentration of ≥400 μg/ml, nor (at 100 μg/ml) did it reduce formazan production in confluent cultures of human fibroblast, Vero, and HeLa cells that had been incubated with XTT (Fig. 4). However, compound S2242 proved to be markedly more cytostatic to the human lymphoblastoid cell lines CEM° and HSB-2 and the murine cell line L1210 than DHPG (Table 2). The 50% effective concentrations (EC_{50}s) for inhibition of [*methyl*-^{3}H]dThd, [5-^{3}H]Urd, and L-[*methyl*-^{3}H]methionine incorporation into HEL cells by compound S2242 were 1.9 ± 1.4, >50, and >50 μg/ml, respectively, in comparison with 18.2 ± 12, >50, and >50 μg/ml, respectively, for ganciclovir. EC_{50}s for inhibition of radiolabelled dThd, Urd, and methionine incorporation in Vero cells by compound S2242 were 3.7 ± 1.3, >50, and 50 μg/ml, respectively, in comparison with 42.7 ± 7.5, >50, and >50 μg/ml, respectively, for ganciclovir.

Duration of antiviral action. Compound S2242, when present for a limited period of time before or after infection in HSV-1, HCMV-, or VZV-infected cell cultures, retained significant antiviral activity. Under the same conditions DHPG and ACV lost virtually all (CMV and HSV) or significant amounts (VZV) of their activity (Table 3).

Inhibitory effects of compound S2242 and ganciclovir on HCMV-induced antigen expression. Neither compound had any inhibitory effect on the expression of IEAs, as determined at 24 h postinfection, even at doses that were completely inhibitory to HCMV replication (>50 μg/ml). By contrast, both compounds efficiently prevented the production of late viral antigen, a process that proceeds only after viral DNA synthesis has taken place. The EC_{50}s for inhibition of late antigen expression by compound S2242 (0.07 ± 0.02 μg/ml) and ganciclovir (0.6 ± 0.6 μg/ml) are close to their EC_{50}s for the inhibition of HCMV induced-cytopathogenicity (Table 1).

Effects of compound S2242 and ganciclovir on HCMV and HSV-1 DNA synthesis. The effects of S2242 and ganciclovir on HCMV DNA synthesis were quantified by CsCl gradient analysis (data not shown). Under our experimental conditions the buoyant densities of cellular DNA and HCMV DNA were 1.68 and 1.70 g/cm^3, respectively. HCMV DNA synthesis was inhibited in a dose-dependent manner, with compound S2242 being more potent than ganciclovir (EC_{50}s, 0.1 and 0.32 μg/ml, respectively). Both S2242 and ganciclovir also effected a concentration-dependent inhibition of HSV-1 DNA synthesis, with ganciclovir (EC_{50}, 0.002 μg/ml) being a more potent inhibitor than compound S2242 (EC_{50}, 0.29 μg/ml). The EC_{50}s for the inhibition of HCMV and HSV-1 DNA synthesis by

TABLE 2. Effect of ganciclovir or compound S2242 on growth of various human and non-human cell lines

Compound	CC_{50} (μg/ml)[a]							
	HEL	HeLa	Vero	C127I	HSB-2	CEM°	FM3A	L1210
S2242	32 ± 2	33 ± 13	12 ± 8.8	26 ± 3	0.6 ± 0.3	1.0 ± 0.3	>100	42 ± 9
DHPG	>100	>50	22 ± 12	36 ± 1	37 ± 2	154 ± 6	77 ± 24	134 ± 3

[a] Data are mean ± standard deviation for at least two to three separate experiments. CC_{50}, concentration required to reduce cell growth by 50%.

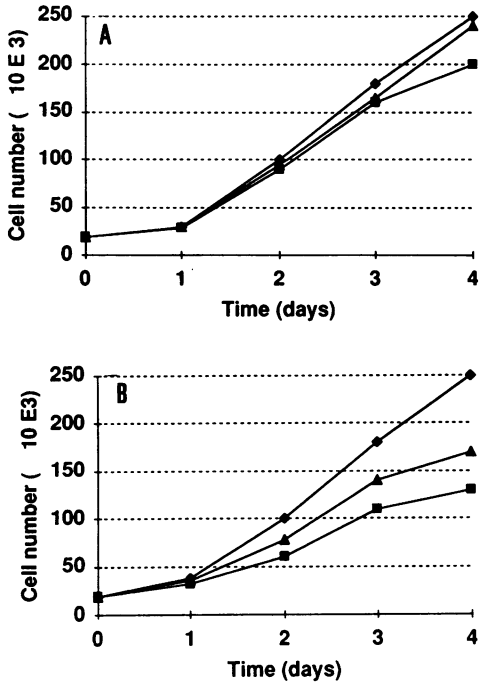

FIG. 2. Effect of ganciclovir (A) or compound S2242 (B) on the growth of uninfected Vero cells over a 4-day period: cell control (◆), 10 μg/ml (■), 5 μg/ml (▲).

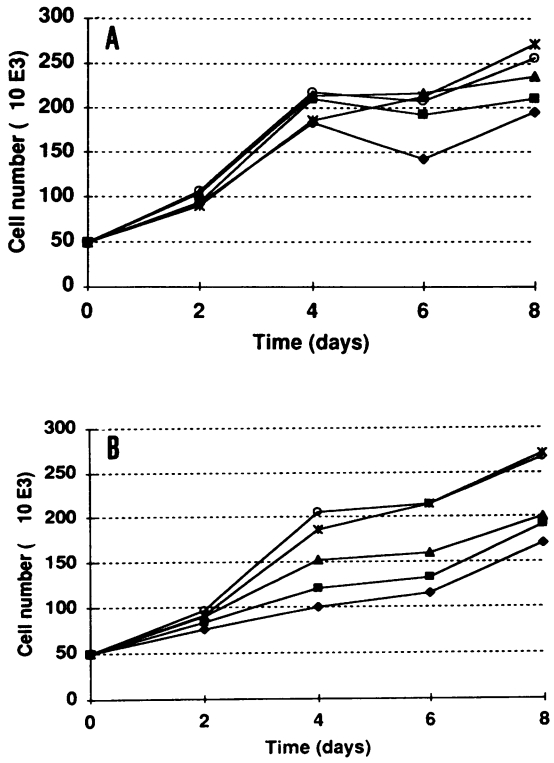

FIG. 3. Effect of ganciclovir (A) or compound S2242 (B) on the growth of uninfected HEL cells over an 8-day period: cell control (*), 50 μg/ml (◆), 12.5 μg/ml (■), 2.5 μg/ml (▲), 0.5 μg/ml (○).

both compounds also correlated with their EC_{50}s for the inhibition of virus-induced cytopathogenicity (Table 1).

Time-of-addition experiment. In order to unravel at which time point in the replication cycle of HCMV or VZV compound S2242 exerts its antiviral effect, time-of-addition experiments were conducted. In a first experiment (Table 4) compound S2242 and DHPG were added to the HCMV-infected cultures at 24-h intervals after infection (from 0 to 144 h postinfection). Thereafter (i.e., at 144 h postinfection), the supernatants from the infected cultures were titrated on HEL cells by determining plaque formation or the induction of IEAs, a process that is not influenced by compound S2242 or DHPG (see above), and thus also not by a possible drug carryover effect. For both compounds, addition to the infected cultures could be delayed for 24 h. When the compounds were first added at 48 h postinfection, a time point at which viral DNA synthesis starts under our experimental conditions (19), culture medium harvested at 144 h postinfection was found to be positive for IEA. Since cell-free VZV is only present in very limited amounts in the supernatants of VZV-infected cultures, we evaluated in a second experiment how long the addition of S2242 to VZV-infected cultures could be delayed before the virus breaks through (data not shown). Akin to DHPG and ACV, addition could be delayed for 48 h; thereafter, the compound lost a significant portion of its antiviral effect (330-fold increase in EC_{50}). Thus, S2242, ganciclovir, and acyclovir appear to interact at similar time points in the replication cycles of HCMV and VZV.

Effects of compound S2242 and ganciclovir on growth of FM3A cells transfected with HSV-1 or HSV-2 TK gene. FM3A TK^-/HSV-1 TK^+ and FM3A TK^-/HSV-2 TK^+ cells are deficient in cytoplasmic TK. The growth of the wild-type murine mammary carcinoma FM3A was not markedly inhibited by DHPG and compound S2242, with the 50% cytostatic concentrations being 77.4 ± 23.8 and >100 μg/ml, respectively. In contrast, the growth of FM3A TK^-/HSV-1 TK^+ and FM3A TK^-/HSV-2 TK^+ cells was inhibited by DHPG at concentrations of 0.25 ± 0.06 and 0.063 ± 0.019 μg/ml, respectively, which is thus 300- to 1,200-fold less than the concentration required to inhibit the growth of the wild-type cells. In contrast, the 50% cytotoxic concentrations of compound S2242 were >100 μg/ml for the HSV-1 TK-transfected cells and 40.6 ± 4.7 μg/ml for the HSV-2-transfected cells.

Effect of dThd on anti-HSV-1 activities of compound S2242, ganciclovir, and ACV. dThd (100 μg/ml) had only a weak reversing effect on the anti-HSV-1 activity of S2242 (EC_{50}s, 0.18 and 0.9 μg/ml in the absence or presence of dThd, respectively, i.e., a fivefold increase in comparison with increases of >400 and 1,500 for ACV and DHPG, respectively).

TK assay. Radiolabelled S2242 was incubated (in the appropriate reaction mixture) with HSV-1-encoded TK derived from FM3A TK^-/HSV-1 TK^+ cells. No metabolites of S2242 were formed (as evaluated by high-pressure liquid chromatographic analysis) after an 1-h incubation period at 37°C.

DISCUSSION

The N-7-substituted acyclic nucleoside analog S2242 was shown to be a potent inhibitor of a wide array of DNA viruses but had no effect on the replication of several RNA viruses or retroviruses. The compound exhibited particularly strong antiherpesvirus activity. Of particular interest is the finding that S2242 is about equally effective against TK-deficient strains of HSV and VZV as against the wild-type strains and that it is

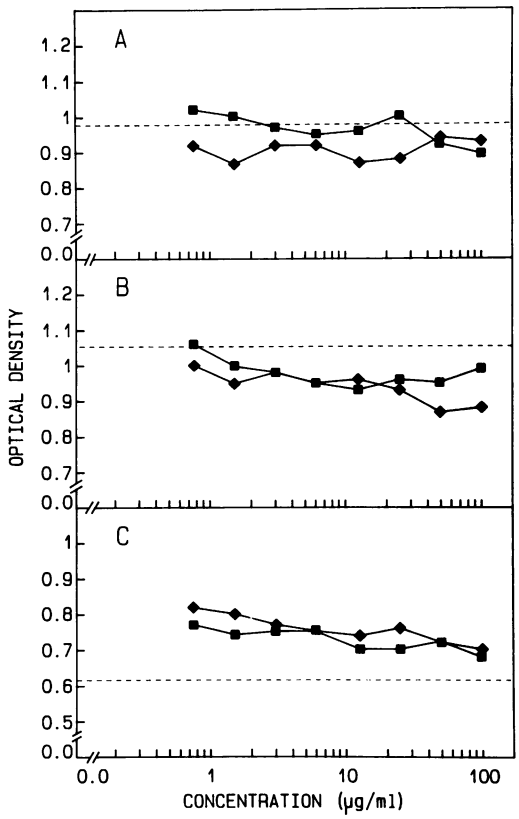

FIG. 4. Effects of compound S2242 (■) and ganciclovir (◆) on formazan production in confluent HeLa (A), Vero (B), and MRC-5 cells (C) after a 20-h drug incubation period at 37°C and a 4-h staining period with XTT. Dashed lines indicate optical density values for the control cultures.

more effective than DHPG against HCMV. From this finding, together with the observations that (i) dThd does not readily reverse the anti-HSV activity of compound S2242, (ii) compound S2242, unlike DHPG, is virtually noncytocidal to FM3A cells transfected with the HSV-1 or HSV-2 TK gene, and (iii) radiolabelled compound S2242 is not (detectably) phosphorylated by HSV-1-encoded TK, it can be concluded that the compound does not (markedly) depend on a viral TK for its activation. From preliminary experiments it appears that compound S2242 elicits activity against DHPG- and ACV-resistant HSV-1 strains (in which drug resistance is due to a defect in the TK gene) and against HCMV strains resistant to DHPG (because of a deficiency in DHPG-phosphorylating activity). On the other hand, compound S2242 appeared to be less effective against foscarnet-resistant HSV-1 strains. Further studies are ongoing to determine the exact resistance profile of compound S2242.

TABLE 4. Effects of S2242 and DHPG on HCMV yield when added at different times after infection

Time of drug addition following virus infection (h)	S2242		DHPG	
	Titer (PFU/ml)[a]	IEA[b]	Titer (PFU/ml)	IEA
0	<10	0	<10	0
24	<10	0	<10	0
48	<10	7	<10	9
72	<10	30	3.2×10^2	17
96	<10	148	5.4×10^3	50
120	1.6×10^3	70	1.0×10^4	100
144	7.5×10^3	360	5.0×10^4	260
Control	8.1×10^4	250		

[a] Virus titer determined by titration on HEL cells.
[b] Induction of IEA expression in the culture medium of HEL cell cultures as evaluated at 24 h postinfection by immunofluorescence microscopy and immunostaining with monoclonal antibody E13. Data represent the number of antigen-positive cells in 10 microscopic fields.

In order to unravel the mechanism of antiherpesvirus activity of compound S2242, we first determined the effect of the compound on the expression of viral (HCMV) antigens. Compound S2242, akin to DHPG, had no inhibitory effect on the expression of IEAs of HCMV but efficiently inhibited the expression of late viral antigens, a process that proceeds after the onset of viral DNA synthesis. By using CsCl gradient analysis it was demonstrated that S2242 inhibits HCMV and HSV-1 DNA synthesis in a concentration-dependent manner and at concentrations which are much lower than those required to inhibit the growth of uninfected cells. The inhibitory concentrations for HSV and HCMV DNA synthesis were comparable to those for the inhibition of viral cytopathogenicity. From this finding it may be assumed that S2242, akin to DHPG, is an inhibitor of viral DNA synthesis. Furthermore, from time-of-addition experiments, it could be deduced that ACV, DHPG, and compound S2242 interfere at a similar time point in the replication cycle of VZV or HCMV, i.e., the time at which viral DNA synthesis starts.

A striking feature of compound S2242 is the persistence of the antiviral effect in vitro after removal of the compound. Such a long-lasting antiviral effect has also been noted for

TABLE 3. Anti-HCMV and anti-VZV activities of compound S2242, DHPG, and ACV following short incubation periods with cells

Time (h) drug present between:	EC_{50} (μg/ml)[a]								
	HCMV			VZV[b]			HSV		
	DHPG	ACV	S2242	DHPG	ACV	S2242	DHPG	ACV	S2242
24 and 0 before infection	>50	>50	0.85 ± 0.20	≥50	>50	0.44 ± 0.19	>50	>50	0.7 ± 0.4
6 and 0 before infection	>50	>50	4.05 ± 1.4	>50	>50	0.92 ± 0.18	>50	>50	1.3 ± 0.2
0 and 24 postinfection	≥16	>50	1.0 ± 0.6	0.46 ± 0.12	1.06 ± 0.26	0.03 ± 0.01			
Continuous[c]	1.4 ± 0.4	18 ± 6.5	0.11 ± 0.07	0.29 ± 0.27	0.11 ± 0.06	0.009 ± 0.001	0.007 ± 0.0005	0.03 ± 0.01	0.45 ± 0.38

[a] Data are means ± standard deviations values for two to four separate experiments. EC_{50}, concentration required to inhibit virus-induced cytopathicity or plaque formation by 50%.
[b] Mean values for the OKA and YS strains.
[c] Five days for VZV, 7 days for HCMV, and 2 to 3 days for HSV.

several acyclic nucleoside phoshonate analogs (HPMPC, PMEA) and for penciclovir but not for antiviral drugs such as DHPG, ACV, 1-(2′-deoxy-2′-fluoro-β-D-arabinofuranosyl)-5-iodocytosine, BVDU, PFA. For the nucleoside phosphonates and penciclovir the persistence of the in vitro activity appeared to correlate with the persistence of drug metabolites intracellularly (9, 19, 28, 29). For the acyclic nucleoside phosphonate analogs this long-lasting antiviral activity has also been observed in several animal models for virus infections as well as in the clinical setting (14, 15, 20, 25–27). However, although S2242 has a more pronounced in vivo activity than DHPG and ACV against several herpesvirus infections, it does not seem to cause as prolonged an antiviral response in vivo as the acyclic nucleoside phosphonate analogs (17).

In conclusion, compound S2242 appears to be a potent and selective inhibitor of herpesvirus replication, with equipotent activity against TK-deficient HSV-1 and VZV strains and wild-type HSV-1 and VZV strains. The viral TK is most likely not involved in the activation of the compound in HSV- or VZV-infected cells. Its mode of action appears to be based on a selective inhibition of viral DNA synthesis. Akin to the acyclic nucleoside phosphonate analogs, the compound confers a long-lasting antiviral effect in vitro. Further studies are required to unravel the intracellular metabolism of this compound and its exact mechanism of antiviral action.

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