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Comparison of antiviral compounds against human herpesvirus 6 and 7

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Abstract

Four classes of antiviral compounds were evaluated for inhibitory activity against two variants of human herpesvirus 6 (HHV-6A and -6B) and human herpesvirus 7 (HHV-7). These included: (1) a pyrophosphate analog, phosphonoformic acid (PFA); (2) beta-guanine analogs, 9-(2-hydroxyethoxymethyl)guanine (acyclovir or ACV), 9-[(1,3-dihydroxy-2-propoxy)methyl]guanine (ganciclovir or GCV) and 9-(4-hydroxy-3-hydroxy-3-hydroxymethyl-butylyl)guanine (penciclovir or PCV); (3) acyclic nucleoside phosphonates, (*S*)-1-[(3-hydroxy-2-phosphonylmethoxy)propyl]cytosine [cidofovir or (*S*)-HPMPC] and its cyclic derivative (*S*)-cyclic-HPMPC (cHPMPC), 9-[[2-hydroxy-1-phosphonomethoxy)ethoxy]methyl]guanine (HPMEMG) and 9-[(2-phosphonylmethoxy)ethyl]-2,6-diaminopurine (PMEDAP), and the seven other related compounds; and (4) a series of benzimidazole ribonucleosides, including 2-bromo-5,6-dichloro-1-(beta-D-ribofuranosyl)benzimidazole (BDCRB). End-point inhibitory concentration (EPC) and 50% effective inhibitory concentration (EC_{50}) values were determined by a dot-blot antigen detection method in cord blood mononuclear cells infected with HHV-6A, HHV-6B or HHV-7 at a multiplicity of infection of 0.004 $CCID_{50}$/cell. (*S*)-HPMPC and cHPMPC had an EC_{50} value of approximately 0.3 μg/ml for HHV-6A, 1.2 μg/ml for HHV-6B and 3.0 μg/ml for HHV-7. These compounds were the most active of those tested against each virus. The EC_{50} value of GCV for HHV-6A was 0.65 μg/ml, 1.33 μg/ml for HHV-6B, and >7 μg/ml for HHV-7. The EC_{50} values of ACV and PCV were approximately 6–8 μg/ml for HHV-6A, 16–24 μg/ml for HHV-6B and 121–128 μg/ml for HHV-7. These drugs were the least active. The sensitivity of HHV-7 to the guanine analogs was different from HHV-6, suggesting a difference in selectivity of specific viral enzymes.

Keywords: Antiviral compounds; Benzimidazole ribonucleosides; Dot-blot method; Human herpesvirus 6; Human herpesvirus 7; Virus replication

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1. Introduction

In recent years, several emerging viruses have been discovered, including human herpesvirus 6 (HHV-6) (Salahuddin et al., 1986, Lopez et al., 1988, Yamanishi et al., 1988, Pellett and Black, 1996) and human herpesvirus 7 (HHV-7) (Frenkel et al., 1990). HHV-6 was first isolated from peripheral blood lymphocytes of patients with acquired immune deficiency syndrome (AIDS) (Salahuddin et al., 1986), from patients with lymphoproliferative disorders and children with exanthema subitum (Lopez et al., 1988, Yamanishi et al., 1988). HHV-7 was first isolated from peripheral blood lymphocytes (Frenkel et al., 1990) and saliva of healthy adults (Wyatt and Frenkel, 1992, Black et al., 1993, Frenkel and Roffman, 1996, Takahashi et al., 1997b). HHV-6 and HHV-7 have been classified as beta herpesviruses.

HHV-6 has been divided into two variants, HHV-6A and HHV-6B, following molecular and epidemiological characterization (Ablashi et al., 1991, Aubin et al., 1991, Inoue et al., 1993, Pellett and Black, 1996). Clinically, HHV-6B is the most common etiologic agent of exanthema subitum (roseola). Diseases caused by HHV-6A are less apparent. Recently, HHV-7 has been reported as another cause of exanthema subitum (Tanaka et al., 1994). While diseases caused by HHV-6 and HHV-7 in childhood are usually not fatal, complications of exanthema subitum (Asano et al., 1990, Kondo et al., 1993, Torigoe et al., 1996, 1997) and life-threatening diseases (e.g. organ transplant, pneumonitis, multiple sclerosis, encephalitis) (Okuno et al., 1990, Asano et al., 1991, Challoner et al., 1995, Knox et al., 1995, McCullers et al., 1995) have been reported.

The differences in biological properties of HHV-6 and HHV-7 (Frenkel and Roffman, 1996, Pellett and Black, 1996, Isomura et al., 1997) assess their sensitivity to antiviral drugs. The antiviral activity of various drugs against these agents has been reported (Streicher et al., 1988, Agut et al., 1989, Åkesson-Johansson et al., 1990, Burns and Sandford, 1990, Williams, 1992, Reymen et al., 1995, Black et al., 1997). Takahashi et al. (1997a) compared the antiviral activity of several drugs against these viruses with an ELISA method. We standardized the assay methodology in order to define the sensitivity of HHV-6 and HHV-7 to several classes of antiviral compounds.

In order to evaluate differences in the sensitivities of HHV-6 and HHV-7, four classes of antiviral compounds were studied. The four classes included:

(1) Phosphonoformic acid (PFA), a non-nucleoside pyrophosphate analog, is a non-competitive inhibitor of viral DNA replication, acting at the pyrophosphate binding site of DNA polymerase (Crumpacker, 1992, Hirsch et al., 1996).

(2) 9-(2-Hydroxyethoxymethyl)guanine (ACV), 9-(4-hydroxy-3-hydroxy-3-hydroxymethylbutylyl) guanine (PCV) and 9-[(1,3-dihydroxy-2-propoxy)methyl]guanine (GCV) are representative guanine analogs which require virus-specific enzymes (i.e. thymidine kinase and UL97 phosphotransferase) for activation (Vere Hodge and Cheng, 1993, Crumpacker, 1996, Hirsch et al., 1996).

(3) (*S*)-1-[(3-Hydroxy-2-phosphonylmethoxy) propyl]cytosine [(*S*)-HPMPC] is a representative acyclic nucleoside phoshphonate which has a phosphonate group on an alkyl side-chain (De Clercq, 1993).

(4) 2-Bromo-5,6-dichloro-1-(beta-D-ribofuranosyl)benzimidazole (BDCRB) is one of benzimidazole ribofuranosides that do not require phosphorylation for antiviral activity (Townsend et al., 1995, Saluja et al., 1996).

Some of these compounds are potent inhibitors of herpes simplex virus (HSV), human cytomegalovirus (HCMV) and varicella zoster virus (VZV) (Faulds and Heel, 1990, Whitley and Gnann, 1992, Wagstaff et al., 1994, Townsend et al., 1995, Hirsch et al., 1996, Saluja et al., 1996).

2. Materials and methods

2.1. Host cells

Fresh cord blood mononuclear cells (CBMCs) were prepared by centrifugation through Histopaque-1077 (Sigma Chemical Co., St. Louis, MO)

and cultured for 3 days in RPMI-1640 medium containing 10% heat inactivated fetal bovine serum, 0.1 U/ml of recombinant human interleukin 2 (GIBCO BRL Life Technology Inc., Grand Island, NY) and 5 μg/ml of phytohemagglutinin (PHA; Sigma Chemical Co., St. Louis, MO) at 37°C in a 5% CO_2 incubator. After 3 days, CBMCs were infected with virus and cultured in RPMI-1640 medium containing the above reagents.

2.2. Preparation of virus stocks

The Z29 strain of HHV-6B (Lopez et al., 1988), U1102 strain of HHV-6A (Downing et al., 1987) and the SB (Black et al., 1993) and RK strains (Frenkel et al., 1990) of HHV-7 were kindly provided by Dr Phillip E. Pellett, Centers for Disease Control and Prevention, Atlanta, GA. The HST (Yamanishi et al., 1988), ADACHI and KYOGOKU strains of HHV-6B and GS strain of HHV-6A (Ablashi et al., 1991) were provided by Dr K. Yamanishi, Osaka University Faculty of Medicine, Osaka, Japan. The MSO strain of HHV-7 was isolated from saliva of a healthy adult.

Cells infected with each virus were co-cultivated with uninfected cells at ratio of 1:5 for 7 days. Virus stocks were prepared by centrifugation of culture fluids at 3000 rpm for 10 min and stored at -80°C. Titration of virus stocks was performed by an end-point dilution method using a dot-blot assay (Yoshida et al., 1996). CBMCs (25 μl, adjusted to 5×10^6 cells/ml) were divided into each well of a 96-well microtiter U-plate. The CBMCs were then infected with 25 μl of virus preparation (in 10-fold dilution series) and incubated for 7 days at 37°C in a 5% CO_2 incubator. After incubation, the supernatant medium was removed and the cells were washed with phosphate-buffered saline (pH 7.4). A dot-blot assay to detect viral antigens is described below. After treatment with Lumi-Phos 530, the membrane was exposed to Fuji RX-U film. The $CCID_{50}$ (50% cell culture infectious dose) was calculated according to the method of Reed and Muench (1938).

2.3. Compounds and monoclonal antibodies

Eleven acyclic nucleoside phosphonates were provided by Gilead Sciences, Foster City, CA, as follows: 9-(2-phosphonylmethoxyethyl)adenine (PMEA), 9-(2-phosphonylmethoxyethyl)-2,6-diaminopurine (PMEDAP), (*S*)-1-[(3-hydroxy-2-phosphonylmethoxy)propyl]cytosine ((*S*)-HPMPC), (*S*)-cyclic-HPMPC (cHPMPC), (*R*)-9-(2-phosphonylmethoxypropyl)adenine [(*R*)-PMPA], D4-API, (*S*)-9-(3-hydroxy-2- (PMEG), oxa-PMEG, 9-[(2-phosphonylmethoxy)methoxymethyl]guanine (PMMMG), 9-[2-hydroxy-1-(phosphonylmethoxy)ethoxy]methyl]guanine (HPMEMG) (Snoeck et al., 1988, Neyts et al., 1990, Kim et al., 1991, Ho et al., 1992, De Clercq, 1993). Ten benzimidazole derivatives were provided by Glaxo Wellcome Co., Research Triangle Park, NC. For comparison, PFA (Sigma Chemical Co.), GCV, ACV (the former Burroughs Wellcome Co., Research Triangle Park, NC) and PCV (Smith Kline Beecham Pharmaceuticals, King of Prussia, PA) were also evaluated.

Monoclonal antibodies (mAbs) to HHV-6 and HHV-7 were established in our laboratory (Tsukazaki et al., 1998) and characterized by radio-immunoprecipitation. For monitoring HHV-6 replication, a mAb (TK-2) recognizing a 135-kDa late polypeptide was used. TK-2 reacts to both variants of HHV-6. For HHV-7, a mAb (IK-3) recognizing a 125-kDa corresponding polypeptide was used. IK-3 is specific to HHV-7.

2.4. Dot-blot method for evaluating the inhibitory effects of compounds to HHV-6 and HHV-7

2.4.1. Infection and addition of compounds

CBMCs were infected with one of the virus strains of HHV-6 or HHV-7 at a multiplicity of infection (MOI) of 0.004 $CCID_{50}$/cell and treated with 10-fold or 2-fold dilutions of a compound. Then 50 μl of medium containing serial dilutions of the compound ($2\times$ concentration) or without compound (control) were added to each well of a 96-well microtiter U-plate. CBMCs (5×10^6 cells/ml) were mixed with the virus dilution (2×10^4 $CCID_{50}$/ml, equivalent to an MOI of 0.004) or

medium only (uninfected control) at a ratio of 1:1. Then 50 μl of infected or uninfected suspension, as prepared above, were added to each well. The microtiter plate was centrifuged at 2000 rpm for 1 h (Pietroboni et al., 1989) and 100 μl of medium containing serial dilutions of the compound was added to each well of the plate. Finally, the plate was incubated for 7 days at 37°C in a 5% CO_2 incubator.

2.4.2. Dot-blot assay to detect viral antigens

To monitor the virus growth in each well, a dot-blot antigen detection was performed, as described previously (Yoshida et al., 1996). After 7 days of incubation, the microtiter plate was centrifuged at 1500 rpm for 10 min. The supernatant medium was removed and the cells were washed with phosphate-buffered saline (pH 7.4). To each well was added 180 μl of lysis buffer (20 mM Tris, 0.5 M NaCl and 0.5% Nonidet-P40, pH 7.5). The cell lysates were spotted onto a nylon membrane (Boehringer Mannheim Biochemica, Indianapolis, IN) through a dot-blot apparatus. The nylon membrane was washed, treated with blocking solution and incubated with a designated mAb overnight at 4°C. The membrane was washed and incubated with anti-mouse IgG alkaline phosphatase conjugate (Sigma Chemical Co.). Finally, the membrane was washed and treated with Lumi-Phos 530 (Boehringer Mannheim). Signals obtained from a dot-blot were analyzed by the Molecular Imager (GS-525 model; Bio. Rad. Co.) with Molecular Analyst for Macintosh software. The end-point concentration (EPC) of each compound was visually determined on a scanning image of the GS-525 model. The EC_{50} (50% effective inhibitory concentration) was calculated using Molecular Analyst.

2.5. Cytotoxicity assay

To determine the cytotoxicity of the compounds, the Alamar Blue assay (Alamar Biosciences Inc., Sacramento, CA) was performed as described previously (Yoshida et al., 1996). Specifically, this assay system incorporates an oxidation–reduction indicator that fluoresces in response to chemical reduction of growth medium resulting from cell growth (Fields and Lancaster, 1993). CBMCs (50 μl, 2.5×10^6 cells/ml) were added to each well of a 96-well microtiter U-plate. Then 50 μl of medium containing 2 $\times$ concentration of each compound at various dilutions was added to each well of the microtiter plate except for untreated control wells where the same volume of medium (without compounds) was added. The plate was incubated at 37°C for 6 days in a 5% CO_2 incubator; 10 μl of Alamar Blue was added to each well and incubated for 24 h. Finally, the microtiter plate was read spectrofluorometrically and the CC_{50} (50% cytotoxic concentration against the growth of CBMCs) was calculated.

3. Results

3.1. Antiviral screening for acyclic nucleoside analogs

The EPC, CC_{50} and selectivity index (SI) of the acyclic nucleoside phosphonates against the Z29 strain of HHV-6B are summarized in Table 1. The EPC values were first determined by 10-fold dilutions of compounds and, more precisely, by 2-fold dilutions for selected compounds. The EPC values of (*R*)-PMPA and D4API were 100 μg/ml, being the least effective of the tested compounds. The EPC for PMMMG was >10 μg/ml. (*S*)-HPMPA and PMEG had EPC values of 1 μg/ml, but the respective cytotoxicities were high. Based on SI values >10, we selected four acyclic nucleoside phosphonates [(*S*)-HPMPC, cHPMPC, PMEDAP, HPMEMG] for more precise assessment of the inhibitory concentrations against HHV-6A, HHV-6B and HHV-7.

The CC_{50} values for PFA and three guanine analogs (ACV, PCV, GCV) were >200 μg/ml. While the CBMCs were heterogeneous in origin, the results of repeated experiments were consistent. Four selected acyclic nucleoside phosphonates were more cytotoxic than PFA and the guanine analogs. Specifically, (*S*)-HPMPC, cHPMPC and HPMEMG were less cytotoxic than PMEDAP, although the range of cytotoxicity was broad.

3.2. Antiviral screening of benzimidazole derivatives

The EPC and CC_{50} of benzimidazole derivatives against the Z29 strain of HHV-6B and the SB strain of HHV-7 were assessed. These compounds, with the EPC values equivalent to >50 μM (≈ 20 μg/ml), were less active against HHV-6 and HHV-7 than the selected acyclic nucleoside phosphonates (Table 2).

3.3. Inhibitory effects of compounds on the replication of HHV-6

Three classes of antiviral compounds were assessed in detail for inhibition of HHV-6A and HHV-6B, including: (1) PFA, (2) ACV, PCV and GCV, and (3) (*S*)-HPMPC, cHPMPC, PMEDAP and HPMEMG.

Table 3 illustrates the inhibitory effects of eight compounds against the GS and U1102 strains of HHV-6A. The EC_{50} values for (*S*)-HPMPC and cHPMPC (≈ 0.3 μg/ml) were lower than those of GCV, PMEDAP (≈ 0.6 μg/ml) and HPMEMG (1.98 μg/ml). Thus, the acyclic nucleoside phosphonates and GCV inhibited the HHV-6A replication more effectively than the other study compounds PFA, ACV and PCV which had the EC_{50} values of >6 μg/ml.

Table 4 illustrates the inhibitory effects of these compounds against HHV-6B. Among the HHV-6B strains, ADACHI and KYOGOKU are recent isolates from patients with exanthema subitum; the other viruses were serially passaged laboratory strains. The EC_{50} values for GCV, (*S*)-HPMPC and cHPMPC (≈ 1.25 μg/ml) were lower than those for PMEDAP and HPMEMG (≈ 2.5 μg/ml). As with HHV-6A, the acyclic nucleoside phosphonates and GCV were more effective against HHV-6B than the other study compounds (PFA, ACV, PCV). While the EC_{50} values of HHV-6B appeared higher than those of HHV-6A, they were not statistically significant (Mann–Whitney *U*-test, $P > 0.05$).

Table 1
Inhibitory effect of acyclic nucleoside analogs on the replication of the Z29 strain of HHV-6B

Compound	EPC (μg/ml)	CC_{50} [a] (μg/ml)	SI [b]
(*S*)-HPMPC	1.0 [c]	63.88 ± 34.70	64
cHPMPC	1.0 [c]	65.67 ± 37.44	66
PMEA	8.0 [c]	46.60 ± 21.11	5.8
PMEDAP	2.0 [c]	20.17 ± 11.39	10
(*R*)-PMPA	100.0	131.80 ± 71.56	1.3
D4API	100.0	31.40 ± 17.59	0.3
(*S*)-HPMPA	1.0	1.87 ± 2.90	1.9
PMEG	1.0	0.51 ± 0.53	0.5
Oxa-PMEG	4.0 [c]	29.6 ± 3.78	7.4
PMMMG	>10.0	93.00 ± 25.27	9.3
HPMEMG	2.0 [c]	103.50 ± 26.35	52
PFA	16.0 [c]	>200	>13
ACV	16.0 [c]	>200	>13
PCV	8.0 [c]	>200	>25
GCV	1.0 [c]	>200	>200

[a] Data shown represent the mean values (± S.D.) of at least five separate experiments.
[b] Selectivity index caluculated as CC_{50}/EPC.
[c] EPC value determined in 2-fold dilutions of the compound.

Table 2
Inhibitory effect of benzimidazole ribonucleosides on the replication of the Z29 strain of HHV-6B and the SB strain of HHV-7

Compound	EPC [a] (μg/ml)		CC_{50} [a,b]
	HHV-6B	HHV-7	
3858W92U*D	>43	≈21.5	>43
3322W93U*C	>39	>19.5	>39
4769W94U*A	>49	>24.5	>49
1254W94U*A	>37	>18.5	>37
2916W93U*C	>39	>19.5	>39
3623W94U*B	>41	>20.5	>41
1038U90U*J (BDCRB)	>35	>35	>70
3542W94U*A	>17.5	>17.5	>35
3212W94U*A	>19	>19	>38
3142W92U*C	>20	>20	>40
1263W94U*G	>19	>19	>38
GCV	1	8	>200

[a] EPC and CC_{50} values were obtained from two separate experiments.
[b] Each value in this column except GCV is equivalent to 100 μM.

Table 3
Antiviral activities of acyclic nucleoside analogs against the replication of HHV-6A

Compound	Virus strains				Range of EPC [a] (μg/ml)	Mean of EC_{50} [b] (μg/ml)	SI [c]
	GS		U1102				
	EPC (μg/ml)	EC_{50} (μg/ml)	EPC (μg/ml)	EC_{50} (μg/ml)			
PFA	16	14.5	16	8	16	11.25 ± 4.60	18
ACV	8	7	16	5	8–16	6.00 ± 1.41	33
PCV	8	8.5	8	7.6	8	8.05 ± 0.64	25
GCV	0.5	0.7	0.5	0.6	0.5	0.65 ± 0.07	308
(*S*)-HPMPC	0.25	0.125	0.5	0.43	0.25–0.5	0.28 ± 0.22	228
cHPMPC	0.25	0.25	0.5	0.44	0.25–0.5	0.35 ± 0.13	188
PMEDAP	0.5	0.55	1	0.7	0.5–1	0.63 ± 0.11	32
HPMEMG	2	1.25	2	2.7	2	1.98 ± 1.03	52
r [d]	0.9952		0.8507				

[a] The range of EPC obtained from two strains.
[b] The mean value ($\pm$ S.D.) of EC_{50} from two strains.
[c] Selectivity index caluculated as CC_{50}/EC_{50}.
[d] Correlation coefficient between EPC and EC_{50} for each virus strain.

3.4. Comparison of inhibition of HHV-6 and HHV-7

Table 5 demonstrates the inhibition of HHV-7 replication by compound. The EC_{50} values for tested compounds against HHV-6A, HHV-6B and HHV-7 are compared in Fig. 1. The EPC and EC_{50} values of the nucleoside analogs (ACV, PCV, GCV) against HHV-7 were significantly higher than those for HHV-6A and HHV-6B (Mann–Whitney U-test, $P < 0.05$). The EC_{50} value for PMEDAP was 0.94 μg/ml. For the other acyclic nucleoside phosphonates, the EC_{50} values were higher, being about 3 μg/ml for (*S*)-HPMPC and cHPMPC and 5.83 μg/ml for HPMEMG. The EC_{50} value for PFA was 9 μg/ml.

The EPC and corresponding EC_{50} values for each virus strain correlated well with one another (Tables 3–5).

3.5. Selectivity index (SI)

The SI value for each compound exhibited similar trends for both variants of HHV-6. With the exception of PFA and PMEDAP, SI values of the other compounds for both variants of HHV-6 were higher than those for HHV-7. Based on the SI values, the order of efficacy of these compounds in the inhibition of both variants of HHV-6 replication was GCV > cHPMPC, (*S*)-HPMPC > HPMEMG > PMEDAP, PFA, ACV, PCV. In contrast, the order of efficacy for HHV-7 was (*S*)-HPMPC, cHPMPC, PFA, PMEDAP > HPMEMG, GCV > ACV, PCV.

4. Discussion

We developed a dot-blot assay to assess the sensitivity of HHV-6 and HHV-7 to several classes of antiviral compounds. Our results are reproducible and objective by standardizing the MOI and using CBMCs as host cells. The CBMCs allow in vitro propagation of T-lymphotropic viruses, such as HHV-6 and HHV-7. This method, especially visual evaluation of the EPC, is simple and will be applicable to the study of other T-lymphotropic viruses, as well. Recently, Takahashi et al. (1997a) compared the effects of antiviral compounds against HHV-6 and HHV-7 by an ELISA and showed some EC_{50} and CC_{50} values different from those presented in

Table 4
Antiviral activities of acyclic nucleoside analogs against the replication of HHV-6B

Compound	Virus strains								Range of EPC [a] (μg/ml)	Mean of EC_{50} [b] (μg/ml)	SI[c]
	Z29		HST		ADACHI		KYOGOKU				
	EPC (μg/ml)	EC_{50} (μg/ml)	EPC (μg/ml)	EC_{50} (μg/ml)	EPC (μg/ml)	EC_{50} (μg/ml)	EPC (μg/ml)	EC_{50} (μg/ml)			
PFA	32	26	16	12	32	32	32	30	16–32	25.00 ± 9.02	8
ACV	32	40	8	8	32	26	32	20	8–32	23.50 ± 13.30	9
PCV	16	26	8	7.8	16	14	16	16	8–16	15.95 ± 7.56	13
GCV	1	1.25	1	0.7	1	1.6	1	1.75	1	1.33 ± 0.47	150
(*S*)-HPMPC	2	1.7	0.5	0.4	1	1.5	1	1.2	0.5–2	1.20 ± 0.57	53
cHPMPC	2	1.7	0.5	0.4	1	1.7	2	1.2	0.5–2	1.25 ± 0.61	53
PMEDAP	4	3	1	0.8	2	2.5	4	3	1–4	2.33 ± 1.04	9
HPMEMG	4	3.7	2	1.8	4	3.2	4	3	2–4	2.93 ± 0.81	35
r [d]	0.9436		0.9843		0.9909		0.9635				

[a] The range of EPC obtained from four strains.
[b] The mean value ($\pm$S.D.) of EC_{50} from four strains.
[c] Selectivity index caluculated as CC_{50}/EC_{50}.
[d] Correlation coefficient between EPC and EC_{50} for each virus strain.

Table 5
Antiviral activities of acyclic nucleoside analogs against the replication of HHV-7

Compound	Virus strains						Range of EPC [a] (μg/ml)	Mean of EC_{50} [b] (μg/ml)	SI [c]
	SB		RK		MSO				
	EPC (μg/ml)	EC_{50} (μg/ml)	EPC (μg/ml)	EC_{50} (μg/ml)	EPC (μg/ml)	EC_{50} (μg/ml)			
PFA	8	7	16	10	8	10	8–16	9.00 ± 1.73	22
ACV	>128	>128	>128	>128	>128	>128	>128	>128	2
PCV	>128	106	>128	>128	>128	>128	>128	120.67 ± 12.70	2
GCV	>8	5.5	>8	>8	>8	7.5	>8	7.00 ± 1.32	29 (13) [d]
(*S*)-HPMPC	2	1.5	4	5	2	1.8	2–4	2.77 ± 1.94	23
cHPMPC	2	1.4	8	6.2	2	2.5	2–8	3.37 ± 2.52	20
PMEDAP	1	0.75	1	0.78	1	1.3	1	0.94 ± 0.31	22
HPMEMG	4	3.5	>8	>8	8	6	4–8	5.83 ± 2.26	18
r [e]	0.9999		0.9062		0.9635				

[a] The range of EPC obtained from three strains.
[b] The mean value ($\pm$S.D.) of EC_{50} from three strains.
[c] Selectivity index calculated as CC_{50}/EC_{50}.
[d] SI value based on CC_{50}/EPC obtained from two extra experiments with 2-fold concentrations (up to 16 μg/ml).
[e] Correlation coefficient between EPC and EC_{50} for each virus strain.

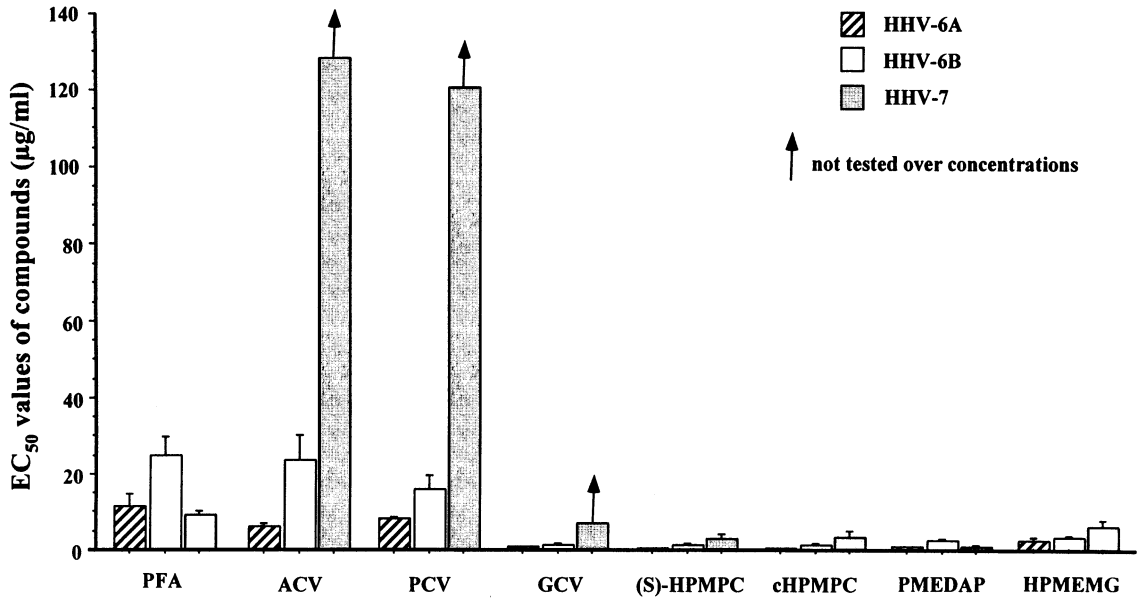

Fig. 1. Comparison of EC_{50} values (mean ± S.E.) of PFA, three beta-guanine analogs and four acyclic nucleoside phosphonates against HHV-6A, HHV-6B and HHV-7.

this study. These discrepancies are likely attributed to assay methodology.

We found that three guanine analogs (ACV, PCV and GCV) had different antiviral activity for HHV-6 and HHV-7. The antiviral activity of these compounds against HHV-7 was extremely low. GCV was effective in vitro against both variants of HHV-6, but the other compounds were less effective for both HHV-6 and HHV-7. These results suggest that these guanine analogs are potentially useful medications for diseases caused by HHV-6 but not for diseases caused by HHV-7.

De Clercq (1993) reported that acyclic nucleoside phosphonates have a broad spectrum of activity against both DNA (HPMPC for HCMV) and RNA viruses (e.g. PMEDAP for human immunodeficiency virus). In this study, as expected, (*S*)-HPMPC and cHPMPC effectively inhibited the replication of both variants of HHV-6. However, higher concentrations of (S)-HPMPC and cHPMPC were required to inhibit HHV-7 than HHV-6. Nevertheless, they were still the most effective compounds for inhibition of HHV-7.

Clinically, GCV can cause bone marrow suppression, particularly neutropenia and thrombocytopenia. Thus, GCV is not an ideal medication for the usually benign clinical disease associated with HHV-6 and HHV-7. On the other hand, for life-threatening disease, such as encephalitis, GCV as well as (*S*)-HPMPC and cHPMPC may prove clinically useful. In fact, (*S*)-HPMPC is licensed for the treatment of HCMV retinitis in several countries.

In this report, differences were demonstrated in the inhibition of HHV-6 and HHV-7 replication using four classes of antiviral compounds which have different mechanisms of action. Interestingly, the concentration of guanine analogs required to inhibit HHV-7 was greater than that for HHV-6. Several investigators have reported that the product of the HCMV UL97 gene phosphorylates GCV to GCV monophosphate, which is subsequently phosphorylated to GCV triphosphate (TP) by host cell enzymes. The GCV-TP competes with dGTP, a natural substrate for the virus-specific DNA polymerase, and inhibits DNA chain elongation (Littler et al., 1992, Lurain

et al., 1994, Metzger et al., 1994, Zimmermann et al., 1997). Sequence analyses of the genomes of HHV-6 and HHV-7 have confirmed that these viruses belong to the beta herpesvirus subgroup along with HCMV. Furthermore, some investigators have reported that the U69 gene of both variants of HHV-6 and HHV-7 is a homologue of the HCMV UL97 gene (Gompels et al., 1995, Nicholas, 1996). Considering these reports, our data suggest that HHV-7 may lack the activity of a virus encoded phosphotransferase which is required for phosphorylation of guanine analogs to the respective monophosphate derivative. In contrast, the replication of HHV-7 was inhibited as effectively as HHV-6 by the acyclic nucleoside phosphonates. Our results suggest a different mechanism of action of these compounds which do not require a virus-specific phosphotransferase (Neyts et al., 1990, Ho et al., 1992, Sullivan et al., 1993).

In the case of benzimidazole ribonucleosides, Townsend et al. (1995) reported that BDCRB, a benzimidazole ribofuranoside, is active against HCMV. Activity and selectivity of BDCRB is superior to GCV or PFA. This compound does not require phosphorylation for antiviral activity and is less toxic than GCV in human bone marrow cells in vitro (Nassiri et al., 1996, Saluja et al., 1996). These reports prompted us to evaluate benzimidazole ribonucleosides against HHV-6 and HHV-7. However, none of the benzimidazole ribonucleosides, including BDCRB, were active against either HHV-6 or HHV-7 in these studies. Considering these results, the mechanism of action of these compounds in the inhibition of HCMV is different than those of HHV-6 and HHV-7. Future studies of differences in the replication will include analysis of specific enzyme activity for HHV-6 and HHV-7.

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