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NOTES

Antiviral Drug Susceptibility of Human Herpesvirus 8

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We studied the susceptibility of human herpesvirus 8 (HHV-8) to a number of antiherpesvirus agents. The acyclic nucleoside phosphonate (ANP) analogs cidofovir and HPMPA [(*S*)-1-(3-hydroxy-2-phosphonylmethoxypropyl)adenine] effected potent inhibition of HHV-8 DNA synthesis, with 50% effective concentrations (EC_{50}) of 6.3 and 0.6 μM, respectively. Adefovir, an ANP with both antiretrovirus and antiherpesvirus activity, blocked HHV-8 DNA replication at a fourfold-lower concentration than did foscarnet (EC_{50} of 39 and 177 μM, respectively). The most potent inhibitory effect was obtained with the N-7-substituted nucleoside analog S2242 (EC_{50}, 0.11 μM). The nucleoside analogs acyclovir, penciclovir, H2G {(*R*)-9-[4-hydroxy-2-(hydroxymethyl) butyl]guanine}, and brivudine had weak to moderate effects (EC_{50} of ≥75, 43, 42, and 24 μM, respectively, and EC_{90} of ≥75 μM), whereas ganciclovir elicited pronounced anti-HHV-8 activity (EC_{50}, 8.9 μM).

Recently a novel virus, Kaposi's sarcoma (KS)-associated herpesvirus, or human herpesvirus 8 (HHV-8), was discovered in an AIDS-KS lesion (3). HHV-8 is a gamma herpesvirus related to herpesvirus saimiri. HHV-8 sequences are detected in nearly all KS lesions (human immunodeficiency virus [HIV] related and unrelated) examined so far. Also, serological studies showed evidence that HHV-8 is specifically associated with KS (6). In addition, the virus is associated with a rare B-cell primary effusion (body cavity-based) lymphoma and some forms of Castleman's disease (2, 12).

HHV-8 replicates in mononuclear cells (5), and lytic infection in the KS lesion appears to occur only in a restricted number of cells of the KS lesion (21). Therefore, inhibition of the lytic cycle of HHV-8 may be expected to have little impact on the evolution of established KS. However, after the initial infection with HHV-8 the virus must replicate, at least in the immunodeficient host, thus amplifying the viral load in the body. It is thus conceivable that if (i) one can block the expansion of the HHV-8 population (this may be shortly after infection or at the time that the immunosuppression is sufficiently profound) and (ii) HHV-8 is indeed the etiological factor in KS and primary effusion lymphoma, then the load of HHV-8 may be too low to cause HHV-8-related malignancies or the risk of developing these malignancies may at least be reduced. From this perspective it is thus important to detect HHV-8 positivity as soon as possible following the initial infection and to start anti-HHV-8 therapy promptly with an effective, nontoxic compound. Alternatively, as suggested by Zhong et al. (21), if latent viral infection drives the proliferation of spindle cells in KS and if this stimulus is not sustained or limited by apoptosis, growth of the lesion would depend on the recruitment of newly infected cells.

Morfeldt and Torssander (10) observed that of five patients with HIV-associated KS, three went into long-term remission following foscarnet treatment. However, factors other than foscarnet may have influenced regression of KS. No follow-up study has been published. In a recent study it was found that HHV-8 DNA was not cleared from the peripheral blood mononuclear cells in five patients under foscarnet and/or ganciclovir (GCV) treatment (8). From a retrospective study of a large series of AIDS patients, it was concluded that the risk for KS was slightly increased with acyclovir (ACV) use, minimally affected with GCV use, and significantly decreased ($P < 0.001$) following foscarnet treatment (9). Some decrease in the risk of KS with GCV, but not ACV, was reported by Glesby et al. (7). It thus seems mandatory to collect information on the susceptibility of HHV-8 to existing antiviral drugs. This study not only may have a clinical impact on the therapy of HHV-8-associated lesions but also may provide deeper insight into the mode of replication of this virus.

We here examined the effects of different antiherpes molecules on the replication of HHV-8 in the 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-inducible HHV-8-containing BCBL-1 cell line (18). Exponentially growing BCBL-1 cells (NIH AIDS Research & Reference Reagent Program) were seeded at a density of 300,000 cells/ml in the absence or presence of 30 ng of the tumor promoter TPA (Sigma) per ml. Cultures were incubated with the different antiviral drugs for 7 days (5 ml of fresh medium containing TPA and antivirals was added at day 4), at which time total cellular DNA was extracted. The origin of the compounds is as described previously (1). Ten micrograms of denaturated total cellular DNA of drug-treated BCBL-1 and control cells were blotted onto a nylon membrane (Hybond-N, Amersham) and UV-cross-linked, after which prehybridization was carried out for 1 h at 42°C. The probe was labeled with digoxigenin-dUTP in a PCR; the 5′-to-3′ primer sequence was AGC CGA AAG GAT TCC ACC ATT and TCC GTG TTG TCT ACG TCC AGA, delimiting a 213-bp sequence within the viral capsid antigen region. The probe was gel purified, and hybridization was carried out for 18 h at 42°C with 30 ng of the digoxigenin-11-dUTP labeled probe per ml. The membrane was washed at high stringency (2× SSC [1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate]–0.1% sodium dodecyl sulfate) for 10 min at room temperature followed by two washes of 15 min each in 0.1× SSC–0.1% sodium dodecyl sulfate at 65°C. After incuba-

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TABLE 1. Inhibitory effects of selected compounds on HHV-8 replication[a]

Compound	EC (μM)[b]		CC_{50} (μM)[c]	SI[d]
	50%	90%		
ACV	≥75	<110	≥690	≤9.2
GCV	8.9 ± 3.5	23 ± 27	354 ± 171	39
PCV	43 ± 7.4	119 ± 24	132 ± 54	3.0
BVDU	24 ± 12	≥75	342 ± 105	≥13
S2242	0.11 ± 0.01	0.6 ± 0.5	24 ± 19	244
HPMPC	6.3 ± 1.8	18 ± 9	340 ± 111	54
HPMPA	0.6 ± 0.9	2.6 ± 1.6	191 ± 122	290
PMEA	39 ± 30	90 ± 0.0	82 ± 12	2.1
PFA[e]	177 ± 57	≥449	≥702	≥3.9
H2G	42 ± 31	≥97	471 ± 33	11

[a] Data are mean values for 3 or 4 independent experiments ± standard deviations.
[b] EC_{50} and EC_{90}, concentrations required to reduce HHV-8 DNA synthesis in TPA-stimulated BCBL-1 cells by 50% and 90%, respectively.
[c] CC_{50}, 50% cytotoxic concentrations (concentrations required to reduce the growth of uninduced BCBL-1 cells by 50% [as evaluated over a 4-day period]).
[d] SI, selectivity index (ratio of CC_{50} to EC_{50}).
[e] PFA, foscarnet.

tion in blocking buffer, the filter was incubated with an anti-digoxigenin antibody and conjugated with alkaline phosphatase (anti-digoxigenin-AP and Fab fragments; Boehringer Mannheim), and detection of chemiluminescence was performed by standard methods. Because the antivirals (see above) had little or no effect on the signal in noninduced cells (only 1 to 3% of the BCBL-1 cells spontaneously enter the lytic cycle [10]), the background signal from the uninduced cultures was subtracted from that of the TPA-induced cultures and the 50% and 90% effective concentrations (EC_{50} and EC_{90}, respectively) were calculated by extrapolation from graphic plots.

The HHV-8 DNA content was increased on average 13.3 ± 2.5-fold (mean ± standard deviation) in the TPA-treated cultures compared to uninduced control cultures. As can be derived from Table 1, cidofovir {HPMPC [(*S*)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine]} and HPMPA [(*S*)-9-(3-hydroxy-2-phosphonylmethoxypropyl)adenine] emerged as potent inhibitors of HHV-8 DNA replication, with EC_{50} of 6.3 ± 1.8 and 0.6 ± 0.9 μM, respectively, and reduced the growth of uninduced BCBL-1 cells by 50% only at concentrations of 340 and 190 μM, respectively, resulting in selectivity indices of 54 and 290. Ninety percent inhibition of HHV-8 DNA synthesis was achieved at 18 μM (5 μg/ml) cidofovir and 2.6 μM HPMPA. Figure 1 depicts the dose-dependent inhibition of HHV-8 DNA synthesis by cidofovir as well as the effect of the compound on the growth of uninduced cells. The HPMPA congener adefovir {PMEA [9-(2-phosphonylmethoxyethyl)adenine]}, a compound with both broad-spectrum antiretrovirus and antiherpesvirus activity, also caused marked inhibition of HHV-8 DNA synthesis (EC_{50}, 39 ± 30 μM). In fact, adefovir proved 4.4-fold-more potent as an anti-HHV-8 agent than foscarnet (EC_{50}, 177 ± 57 μM). Adefovir caused 90% inhibition of HHV-8 DNA synthesis at a concentration of 90 μM, whereas foscarnet did so only at concentrations of ≥449 μM. ACV, penciclovir (PCV), brivudine (BVDU), and H_2G {(*R*)-9-[4-hydroxy-2-(hydroxymethyl)butyl]guanine}, compounds that depend for their activation on a virus-encoded thymidine kinase (TK), had weak to moderate inhibitory effects on the lytic replication of HHV-8 (EC_{50} of ≥75, 43, 24, and 42 μM, respectively, and EC_{90} of ≥75 μM). Yet GCV conferred antiviral protection, with an EC_{50} of 8.9 μM (EC_{90}, 23 μM). The novel nucleoside analog S2242 [(1,3-dihydroxy-2-propoxymethyl)purine], which can be viewed as a 6-deoxy form of GCV with the acyclic side chain substituted at the N-7 rather than the N-9 position, emerged as the most potent anti-HHV-8 compound of this series, with an EC_{50} of 0.11 ± 0.01 μM and an EC_{90} of 0.6 ± 0.5 μM. When cidofovir and GCV (≈100 μM) were added to the TPA-induced cell cultures for only a 72-h period, followed by extensive washing of the cultures, cidofovir still caused complete inhibition of HHV-8 DNA synthesis when evaluated 7 days after TPA induction. By contrast, under this experimental condition GCV lost all protective effect (EC_{90}, >100 μM) (data not shown). Very recently, Kedes and Ganem (10) reported on the anti-HHV-8 activity of ACV, GCV, foscarnet, and cidofovir. Antiviral activities similar to those described in the present study for these four compounds were reported.

We next studied the intracellular phosphorylation of ACV or BVDU over a 24-h period in BCBL-1 cells that had been either pretreated for 24 h with 30 ng of TPA per ml or left untreated and that received either 2 μCi of [8-^{3}H]ACV/ml or 0.3 μCi of [2-^{3}H]BVDU/ml. High-performance liquid chromatography analysis did not reveal major differences in the phosphorylation of ACV in either the TPA-induced or uninduced cells (i.e., 1.4 and 2.0 pmol/10^6 cells of ACV monophosphate and 4.0 and 4.4 pmol/10^6 cells of ACV triphosphate in TPA-induced and uninduced cells, respectively). In contrast, ACV was efficiently phosphorylated to its mono-, di-, and triphosphate forms in HSV-1-infected Vero cells but not in mock-infected Vero cells (data not shown). TPA also did not induce major differences in the formation of BVDU mono-, di-, and triphosphate in BCBL-1 cells (i.e., 42, 8.6, and 15.4 pmol/10^6 cells and 28, 5.6, and 6.6 pmol/10^6 cells for TPA-induced cultures and uninduced cultures, respectively).

Cidofovir, a broad-spectrum anti-DNA virus agent that has recently been approved in the United States and Europe for the treatment of cytomegalovirus retinitis in AIDS patients (13), is an acyclic nucleoside phosphonate analog that does not depend on virus-encoded kinases to become antivirally active. In its diphosphorylated form, the compound selectively blocks the viral DNA polymerization process. The anti-HHV-8 activity of cidofovir reported here must depend on the selective inhibitory activity of diphosphorylated HPMPC against the HHV-8 DNA polymerase. HPMPA proved eightfold-more potent than cidofovir but has not been developed clinically. A unique characteristic of cidofovir is its long-lasting antiviral activity (14). In the clinical setting this feature translates into infrequent dosing (19). We also demonstrate here that cidofovir, but not GCV, retains marked anti-HHV-8 activity when

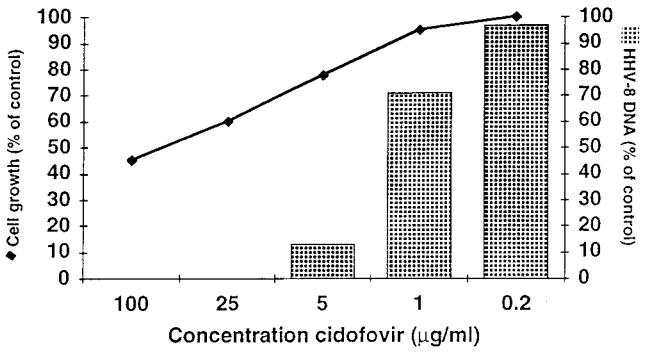

FIG. 1. Effects of cidofovir (HPMPC) on HHV-8 DNA synthesis in TPA-induced BCBL-1 cells and on the growth of uninduced BCBL-1 cells.

added to cell cultures for only a short period of time. High intracellular concentrations of the drug metabolites, even long after their levels in plasma drop below the EC_{50} for virus replication, are responsible for this effect (13). Whereas cidofovir is solely active against DNA viruses, adefovir is also a potent inhibitor of retrovirus and hepadnavirus replication (13). The oral prodrug form of PMEA, bis(pivaloylmethyl) ester (adefovir dipivoxil), is currently undergoing phase II/III trials for anti-HIV and anti-hepatitis B virus activity. Like foscarnet, adefovir inhibits both HIV and HHV-8. However, adefovir inhibits the replication of HHV-8 at a threefold-lower concentration than does foscarnet and causes 90% inhibition of HHV-8 DNA synthesis at a concentration of 90 μM, whereas foscarnet does so only at ≥449 μM. With respect to their antiherpesvirus activities, adefovir and foscarnet show a cross-resistance pattern (20). This suggests that both compounds have similarities in their mechanisms of action. This may be particularly interesting in view of the reported beneficial effect of foscarnet on KS (9, 11). The concentrations of adefovir that are reached in plasma are in the range of the EC_{50} for inhibition of KS-associated herpesvirus replication (4). Anti-HIV therapy with adefovir could thus possibly serve as an anti-HHV-8 prophylaxis.

Neither ACV, PCV, BVDU, nor H2G, molecules that depend for their activation on a viral TK, have pronounced anti-HHV-8 activity. GCV proved more effective, with a three- to eightfold-lower EC_{50}. We were not able, by using the system employed, to detect specific phosphorylation of ACV or BVDU in the TPA-induced BCBL-1 cells. This, together with the weak activity of the compounds against HHV-8, may suggest that these molecules are weak substrates for the HHV-8-encoded TK. Alternatively, or in addition, the triphosphates may be weak inhibitors of the viral DNA polymerase.

S2242 is a novel nucleoside analog that potently inhibits the replication of all herpesviruses tested, including TK-deficient strains (15). The very potent anti-HHV-8 activity of S2242 (0.1 μM) must be related to (i) the fact that the compound is efficiently phosphorylated in lymphoid cells (17) and (ii) possible potent inhibition of HHV-8 DNA polymerase by the S2242 triphosphate metabolite. Although the compound has a rather pronounced cytostatic effect on lymphoid cells, it was very well tolerated and proved highly effective in the treatment of a variety of herpesvirus infections in several animal models (16).

In conclusion, three molecules that are currently in clinical use (cidofovir, ganciclovir, and foscarnet) or under clinical study (adefovir) have marked anti-HHV-8 activity. Moreover, cidofovir has long-lasting activity against HHV-8. Finally, a molecule like adefovir that is used as an anti-HIV agent and that, in addition, has good anti-HHV-8 activity, may be particularly interesting for prophylactic use in the HIV-infected population that is at risk for acquiring HHV-8 infection.

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