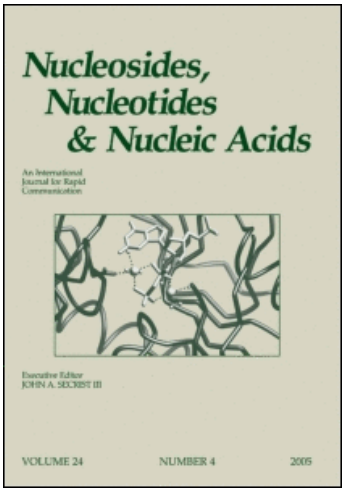

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ANTIVIRAL ACTIVITY OF SELECTED NUCLEOSIDE ANALOGUES AGAINST HUMAN HERPES VIRUS TYPE 6

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ABSTRACT: HHV-6 was evaluated *in vitro* for its susceptibility to a broad range of nucleoside analogues. PFA and several acyclic nucleoside phophonates emerged as the most potent inhibitors of HHV-6 replication as monitored by a newly developed immunofluorescence / flow cytometric assay as well as by microscopical evaluation of their inhibitory effect on HHV-6-induced cytopathogenicity.

INTRODUCTION

Human herpes virus type 6 (HHV-6) was initially isolated from peripheral blood lymphocytes of patients with acquired immune deficiency syndrome (AIDS) or various lymphoproliferative diseases[1]. The etiological role of HHV-6 in nervous system diseases or lymphadenopathies is still debated. HHV-6 may be a co-factor in the pathogenesis of AIDS in HIV-infected patients[2,3]. HHV-6 could also be involved in opportunistic infections associated with immunosuppression, as are the other human herpes viruses.

The present study was aimed at evaluating the *in vitro* sensitivity of HHV-6 to several antiviral compounds, including the acyclic nucleoside phosphonates. This class of broad-spectrum antiviral agents exhibit potent and selective activity against DNA- and/or retroviruses[4].

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MATERIALS AND METHODS

Virus and Cells. The GS strain of HHV-6, originally isolated from a patient with a lymphoproliferative disorder[1], was kindly provided by Dr. D.V. Ablashi (National Cancer Institute, National Institute of Health, Bethesda, MD, USA). Antiviral activity and cytotoxicity assays were performed in human T-lymphoblastoid HSB-2 cells (ATCC CCL 120.1), propagated in RPMI 1640 medium.

Antiviral Activity Assays. HSB-2 cells were infected with HHV-6 and incubated with various concentrations of the test compounds (for abbreviations of compounds: see footnote b to Table 1). Microscopical evaluation of viral cytopathic effect (CPE) (i.e., appearance of large refractile cells) was performed on day 7. An indirect immunofluorescence assay was performed on the same cell cultures. Therefore, the samples were incubated with a high-titer anti-HHV-6 polyclonal antiserum, kindly provided by Dr. G. R. F. Krueger (University of Cologne, Germany). In a second step, the cells were labeled with fluorescein isothiocyanate conjugated $F(ab')_2$ fragments of rabbit anti-human immunoglobulin antibody. Then, the samples were analysed with a fluorescence activated cell sorter.

Cytotoxicity Assay. HSB-2 cells were cultured in the presence of serial dilutions of the test compounds. After 7 days, the number of cells was determined in a Coulter counter.

RESULTS AND DISCUSSION

In order to evaluate the anti-HHV-6 activity of selected nucleoside analogues, we developed an indirect immunofluorescence / flow cytofluorographic method to assess the inhibitory effect of the compounds on HHV-6-induced antigen expression in HHV-6-infected cells. The compounds were also evaluated microscopically for their inhibitory effect on HHV-6-induced cytopathogenicity. There was a close correlation between the antiviral data obtained by these two assays (Table 1). PFA has been found to protect HSB-2 cells against HHV-6 infection at a concentration of approximately 20 μg/ml[5,6]. Under the experimental conditions of our study, PFA emerged as the most potent inhibitor of HHV-6 replication with an EC_{50} of 2.2 μg/ml as monitored by the indirect immunofluorescence / flow cytometric method, and a selectivity index of 125. ACV appeared to have a slight protective effect with an EC_{50} of 70 μg/ml and a selectivity index of only 3. This finding is in agreement with previous reports[5,6]. GCV has been reported to exhibit incomplete

TABLE 1. Inhibitory effect of selected nucleoside analogues on the replication of HHV-6 in HSB-2 cells[a].

Compound[b]	EC_{50}[c] (μg/ml) based on		CC_{50}[d] (μg/ml)	Selectivity index[e]
	CPE	IF		
PFA	4.4 ± 1.8	2.2 ± 0.8	274 ± 23	125
PMEDAP	3.3 ± 1.3	2.8 ± 1.9	51 ± 7	18
PMEA	7.0 ± 3.9	8.5 ± 7.1	48 ± 12	6
(*S*)-HPMPA	3.1 ± 1.1	2.7 ± 0.9	14 ± 3	5
(*S*)-HPMPC	4.6 ± 1.5	3.8 ± 2.9	39 ± 5	10
(*S*)-HPMPDAP	8.1 ± 0.9	12.1 ± 7.3	99 ±43	8
(*S*)-FPMPA	NA[f]	NA	ND[g]	–
(*R*)-PMPDAP	NA	NA	ND	–
ACV	39.8 ± 5.0	69.8 ± 15	204 ± 18	3
GCV	NA	NA	15 ± 2	–
BVDU	NA	NA	0.2	–

[a]All data represent mean values (± standard deviation) of one to four separate experiments.
[b]Abbreviations of test compounds: PFA (foscarnet, phosphonoformic acid); ACV [acyclovir, 9-(2-hydroxyethoxymethyl)guanine); GCV(ganciclovir, 9-(1,3-dihydroxy-2-propoxymethyl)-guanine); BVDU [brivudin, (*E*)-5-(2-bromovinyl)-1-(ß-D-2-deoxyribofuranos-1-yl)uracil]; PMEA [9-(2-phosphonylmethoxyethyl)-adenine]; PMEDAP [9-(2-phosphonylmethoxyethyl)-2,6-diaminopurine]; (*S*)-HPMPA [(*S*)-9-(3-hydroxy-2-phosphonylmethoxypropyl)adenine]; (*S*)-HPMPC [(*S*)-1-(3-hydroxy-2-phosphonylmethoxypropyl)-cytosine]; (*S*)-HPMPDAP [(*S*)-9-(3-hydroxy-2-phosphonylmethoxypropyl)-2,6-diaminopurine]; (*R*)-PMPDAP [(*R*)-9-(2-phosphonylmethoxypropyl)-2,6-diaminopurine] and (*S*)-FPMPA [(*S*)-9-(3-fluoro-2-phosphonylmethoxypropyl)adenine].
[c]Compound concentration required to inhibit viral replication by 50%, based on microscopical evaluation of HHV-6-induced cytopathogenic effect (CPE), or on immunofluorescence / flow cytometric analysis of HHV-6-specified antigen expression (IF).
[d]Compound concentration causing 50% inhibition of cell growth, as measured by cell counting with a Coulter Counter.
[e]Ratio of CC_{50} to EC_{50}.
[f]Not active at subtoxic concentration.
[g]Not determined.

inhibition of HHV-6 replication in HSB-2 cells[5]. However, marked anti-HHV-6 effect was noted in peripheral blood mononuclear cells (PBMC)[6]. In our hands, GCV proved not active at a subtoxic concentration. These conflicting observations could be related to the stringent experimental conditions of our study [high viral load and long (7-day) incubation period of virus-infected cells].

This is the first report on the anti-HHV-6 activity of a number of acyclic nucleoside phophonates. PMEDAP, (*S*)-HPMPA and (*S*)-HPMPC proved to be equally active as PFA with an EC_{50} ranging from 2–4 μg/ml. PMEA and (*S*)-HPMPDAP were effective at an EC_{50} of 8.5 μg/ml and 12.1 μg/ml, respectively; whereas (*S*)-FPMPA and (*R*)-PMPDAP showed no activity at subtoxic concentrations. The highest selectivity indexes were recorded for PMEDAP (18), (*S*)-HPMPC (10) and (*S*)-HPMPDAP (8), followed by PMEA (6) and (*S*)-HPMPA (5).

In conclusion, for the acyclic nucleoside phosphonates, there was a close correlation between their anti-HHV-6 activity and their activity against other herpesviruses (i.e., herpes simplex virus type 1 and 2 and cytomegalovirus)[4,7,8]. The fact that PMEA and PMEDAP are, on one hand, potent inhibitors of human immunodeficiency virus (HIV) and, on the other hand, exhibit potent activity against herpesviruses including HHV-6, makes these compounds of potentially great value in the treatment of AIDS patients suffering from intercurrent herpesvirus infections. We now plan to evaluate the effects of the acyclic nucleoside phophonates on mixed infections of HIV and HHV-6.

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