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Activities of Various Compounds against Murine and Primate Polyomaviruses

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Polyomavirus infections in humans are due to BK virus (BKV) and JC virus (JCV). Diseases associated with human polyomaviruses occur mostly in immunocompromised adults, e.g., progressive multifocal leukoencephalopathy (PML), caused by JCV, in AIDS patients and hemorrhagic cystitis and uretral stenosis, caused by BKV, in transplant recipients. No therapy is available for these diseases, which necessitates the development of chemical entities that are active against polyomaviruses. Several antiviral compounds were evaluated to determine their effects on the in vitro replication of mouse polyomavirus and the primate viruses simian virus 40 (SV40), SV40 PML-1, and SV40 PML-2. The activity of the different compounds was assessed by a cytopathic effect reduction assay and confirmed in a virus yield assay. Cidofovir [HPMPC; (*S*)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine] and its cyclic counterpart emerged as the most selective antipolyomavirus agents. The 50% inhibitory concentrations for HPMPC were in the range of 4 to 7 μg/ml, and its selectivity index varied from 11 to 20 for mouse polyomavirus and from 23 to 33 for SV40 strains in confluent cell monolayers. Cell cytotoxicity was up to 15-fold greater in growing cells. Other acyclic nucleoside phosphonates (i.e., HPMPA; [(*S*)-9-(3-hydroxy-2-phosphonylmethoxypropyl)adenine] and PMEG [9-(2-phosphonylmethoxyethyl)-guanine]) also showed some activity but had low selectivity. None of the other drugs tested against these animal viruses (i.e., acyclovir, ganciclovir, brivudine, ribavirin, foscarnet, and cytarabine) showed significant activity. Thus, HPMPC deserves further evaluation as a candidate drug for polyomavirus infections in the immunocompromised host.

Polyomaviruses are nonenveloped DNA viruses with double-stranded, covalently closed, circular DNA genomes. Polyomaviruses are widely distributed in nature and have been described as occurring in a variety of species, including birds, rodents, rabbits, and primates.

In humans, polyomavirus infection is associated with progressive multifocal leukoencephalopathy (PML), a rare, demyelinating, fatal disorder of the central nervous system, usually occurring in the immunocompromised individual (16, 17). The incidence of PML has increased over the last decade because of the wide spread of human immunodeficiency virus (HIV) infection, leading to immunodeficiency (4, 26). PML can be the initial manifestation of AIDS, and it is estimated to occur in approximately 5% of all AIDS patients. The etiologic agent of PML, JC virus (JCV), causes lytic infection of the myelin-producing oligodendrocytes in the white matter of the brain, which results in focal or multifocal plaques of demyelination. Another human polyomavirus, BK virus (BKV), is associated with hemorrhagic cystitis, urethral stenosis, and some of the urinary tract illnesses in renal allograft recipients and bone marrow transplant patients (2, 3). BKV DNA has also been recovered from human adenomas, pancreatic islet cells, insulomas, gliomas, and other tumors of the brain (5, 9, 21). It is not known whether BKV plays any role in the etiology of these tumors.

Most of the primary infections with BKV and JCV occur in childhood. The viruses persist indefinitely in the infected individual and are reactivated upon impairment of the immune system through HIV infection, neoplastic disease, or drug treatment for graft protection. BKV and JCV are both related to simian virus 40 (SV40), an oncogenic polyomavirus of Asian macaques, as determined by nucleotide sequence homology (10, 21). SV40 was discovered in 1960 as a contaminant in polio vaccines prepared from rhesus monkey kidney cell cultures. SV40 and the mouse polyomavirus are the best characterized of the polyomaviruses. The murine polyomavirus was named for its remarkable ability to induce tumors at multiple sites in its natural host.

There is currently no accepted treatment for JCV and BKV infections, and only the use of cytosine arabinoside (Ara-C; also called cytarabine) has been tried in the largest number of patients. Reports have not shown a consistent positive response (1, 13), but in a few patients with PML, treatment with Ara-C seems to have produced some clinical improvement (20). Treatment with a combination of cytarabine and alpha interferon has been reported to produce a marked improvement in the clinical course of PML (24).

There are no data available on the effect of nucleoside analogs on polyomavirus replication in cell culture. The most susceptible cell types that support the replication of JCV and BKV are primary cultures derived from human embryo tissues. BKV grows well in human embryonic kidney cells and epithelial cells (12), while JCV grows best in human fetal brain cultures and multiplies in astrocytes and the precursor oligodendroglial cells (15). Even under optimum conditions, viral growth may take a few days to several weeks.

The difficulty involved in efficiently propagating BKV and JCV in vitro prompted us to use four strains of murine polyomavirus and three strains of SV40 to assay the inhibitory effect of several antiviral compounds on the in vitro replication of polyomavirus. In the present study, we evaluated several nucleoside analogs, including acyclic nucleoside phosphonate analogs such as (*S*)-1-(3-hydroxy-2-phosphonylmethoxypro-

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TABLE 1. Susceptibility of murine polyomavirus to selected antiviral compounds in UC1-B cells

Compound	CC_{50} (μg/ml)	IC_{50} (μg/ml) for:			
		MN/RDE Toronto	PTA	2PTA2	LID-1
HPMPC	78 ± 17	7.1 ± 3.1	3.9 ± 2.1	5.9 ± 2.6	6.5 ± 2.3
cHPMPC	170 ± 42	15.6 ± 9.0	13.4 ± 11.6	17.9 ± 14.5	21.8 ± 17.5
HPMPA	24 ± 6	7.6 ± 1.9	6.7 ± 4.7	9.3 ± 4.6	9.2 ± 4.3
PMEA	14 ± 9	3.5 ± 2.7	4.8 ± 3.9	4.8 ± 2.1	5.5 ± 4.4
PMEDAP	3.9 ± 2.4	0.5 ± 0.3	0.6 ± 0.2	1.1 ± 0.6	0.9 ± 0.4
PMEG	0.17 ± 0.13	0.029 ± 0.019	0.064 ± 0.023	0.061 ± 0.043	0.076 ± 0.033
ACV	173 ± 46	>50	>50	>50	>50
GCV	180 ± 35	>50	>50	>50	>50
BVDU	183 ± 29	>50	>50	>50	>50
PFA	>200	117.5 ± 6.1	104.2 ± 45.0	143.0 ± 36.7	121.4 ± 57.3
Ara-A	99 ± 87	35.1 ± 13.6	33.9 ± 10.7	44.6 ± 8.0	35.4 ± 10.0
Ribavirin	96 ± 90	>50	>50	>50	>50

pyl)cytosine (HPMPC), which has potent and selective activity against a broad spectrum of DNA viruses (6, 7).

MATERIALS AND METHODS

Cells. UC1-B cells (murine embryo fibroblasts, ATCC 6465-CRL) and BS-C-1 cells (African green monkey kidney cell line ATCC CCL-26) were used. Cells were propagated in minimum essential medium (MEM) supplemented with 10% inactivated fetal calf serum (FCS), 1% L-glutamine, and 0.3% sodium bicarbonate.

Virus strains. Four murine polyomavirus strains were used: MN/RDE Toronto, PTA, 2PTA2, and LID-1 (ATCC VR-252). SV40 (a vacuolating agent) strain A2895 (ATCC VR-305), SV40 PML-1 strain EK (ATCC VR-820), and SV40 PML-2 strain DAR (ATCC VR-821), derived from brain cultures of two patients with PML, were also employed. The polyomavirus strains and the SV40 strains were propagated and assessed in UC1-B and BS-C-1 cells, respectively.

Compounds. The compounds evaluated for their inhibitory effects on the virus isolates were as follows: 9-(2-hydroxyethoxymethyl)guanine, also called acyclovir (ACV), from Wellcome Research Laboratories, Research Triangle Park, N.C.; 9-(1,3-dihydroxy-2-propoxymethyl)guanine (DHPG), also called ganciclovir (GCV), from Syntex, Palo Alto, Calif.; (*E*)-5-(2-bromovinyl)-1-(β-D-2-deoxyribofuranos-1-yl)uracil (BVDU), also called brivudine, from P. Herdewijn, Rega Institute for Medical Research, Leuven, Belgium; ribavirin, also called virazole, from ICN Pharmaceuticals, Costa Mesa, Calif., 9-β-D-arabinofuranosyladenine, also called vidarabine (Ara-A), and phosphonoformate sodium salt, also called foscarnet (PFA), from Sigma Chemical Co., St. Louis, Mo.; 9-(2-phosphonylmethoxyethyl)adenine (PMEA), 9-(2-phosphonylmethoxyethyl)guanine (PMEG), and HPMPC from Gilead Sciences, Foster City, Calif.; (*S*)-9-(3-hydroxy-2-phosphonylmethoxypropyl)adenine (HPMPA), cyclic HPMPC (cHPMPC), and 9-(2-phosphonylmethoxyethyl)-2,6-diaminopurine (PMEDAP) from A. Holý, Institute of Organic Chemistry, Academy of Sciences, Prague, Czech Republic.

Antiviral assays. Confluent UC1-B and BS-C-1 cells grown in 96-well microtiter plates were infected with the different polyomavirus or SV40 strains, respectively, at 100 $CCID_{50}$ (1 $CCID_{50}$ corresponds to the virus stock dilution that is infective for 50% of the cell cultures). After a 2-h incubation period, residual virus was removed, and the infected cells were further incubated with MEM supplemented with 2% inactivated FCS, 1% L-glutamine, and 0.3% sodium bicarbonate containing serial dilutions of the test compounds (in duplicate). After 4 to 5 days (polyomavirus strains) or 6 to 7 days (SV40 strains) of incubation at 37°C in a 5% CO_2 atmosphere, virus-induced cytopathic effect (CPE) was monitored microscopically. CPE in polyomaviruses is recognized by cell rounding and clumping, while SV40 produces enlargement and vacuolization of the cells. Antiviral activity is expressed as the 50% inhibitory concentration (IC_{50}), i.e., the compound concentration required to reduce viral CPE by 50%. IC_{50}s were estimated from graphic plots (on semilogarithmic paper) of percent CPE as a function of the concentration of the test compounds. The IC_{50}s for each compound are the mean IC_{50}s for three or more independent experiments.

Cytotoxicity assays. Confluent monolayers of UC1-B or BS-C-1 cells grown in 96-well microtiter plates were incubated with MEM supplemented with 2% inactivated FCS, 1% L-glutamine, and 0.3% sodium bicarbonate containing different concentrations of the compounds (in duplicate). Cell cultures were incubated for 5 to 6 days, and the cells were trypsinized. The cell number was determined with a Coulter Counter. The 50% cytotoxic concentration (CC_{50}) is the concentration required to reduce cell number by 50% relative to the number of cells in the untreated control cell cultures. The selectivity index is the ratio of CC_{50} for cell growth to IC_{50} for viral CPE.

Determination of the number of cells in control (uninfected) and infected cell cultures. Confluent monolayers of UC1-B or BS-C-1 cells grown in 96-well microtiter plates were left uninfected (controls) or infected with the LID-1 and PTA strains or the SV40 PML-2 strain, respectively, at 100 $CCID_{50}$. Uninfected control cultures were tested in parallel and were treated with medium during the viral adsorption time. After a 2-h adsorption period, the medium (control cultures) or the viral inoculum (infected cultures) was removed, and the infected cells as well as the control uninfected cell cultures were further incubated with MEM supplemented with 2% inactivated FCS, 1% L-glutamine, and 0.3% sodium bicarbonate containing serial dilutions of the test compounds (in duplicate). After 5 days (polyomavirus strains) or 7 days (SV40 PML-2 strain) of incubation at 37°C in a 5% CO_2 atmosphere, virus-induced CPE was monitored microscopically, and immediately thereafter the infected and uninfected control cultures were trypsinized. The cell number was then determined with a Coulter Counter.

Virus yield assays. Confluent cells grown in 6-well microtiter plates were infected with the different strains of polyomavirus or SV40 at a multiplicity of infection of approximately 0.1. After 2 h of incubation, residual virus was removed and replaced by medium containing different concentrations of the test compounds. At various times postinfection, the supernatants were harvested and frozen at −70°C until they were assayed for virus content by titration in 96-well microtiter plates.

Time-of-addition experiments. Confluent UC1-B or BS-C-1 cells were infected as described for the antiviral assays. After 2 h of adsorption, residual virus was removed, and the infected cells were incubated with MEM supplemented with 2% inactivated FCS. At various times postinfection, medium containing different concentrations of HPMPC was added, and the cells were further incubated until viral CPE was recorded (4 to 5 days for polyomaviruses and 7 days for SV40). Viral CPE was then monitored microscopically, and the IC_{50}s were calculated.

RESULTS

Inhibitory effects of various antiviral compounds on murine polyomaviruses. Four murine polyomavirus strains (MN/RDE Toronto, PTA, 2PTA2, and LID-1) were examined for their susceptibility to various antiviral compounds in UC1-B cells. The IC_{50}s of the test compounds for the murine polyomavirus strains and the corresponding selectivity indices are presented in Tables 1 and 2, respectively.

The compounds varied considerably in potency and selectivity. Among the drugs tested, only the acyclic nucleoside phosphonate analogs showed a significant inhibitory effect on the replication of the four different murine polyomavirus strains. Among the acyclic nucleoside phosphonate analogs, HPMPC and cHPMPC emerged as the most selective inhibitors of murine polyomavirus-induced CPE. The IC_{50}s for HPMPC and cHPMPC were in the range of 4 to 7 and 13 to 22 μg/ml, respectively, while their selectivity indices ranged from 11 to 20 and 8 to 11, respectively.

The IC_{50}s for HPMPA, PMEA, PMEDAP, and PMEG ranged from 6.7 to 9.3, 3.5 to 5.5, 0.5 to 1.1, and 0.029 to 0.076 μg/ml, respectively. These IC_{50}s were close to the corresponding CC_{50}s (24, 14, 3.9, and 0.17 μg/ml for HPMPA, PMEA, PMEDAP, and PMEG, respectively), which resulted in low

TABLE 2. Selectivity indices of antiviral compounds in UC1-B for murine polyomavirus

Compound	Selectivity index for:			
	MN/RDE Toronto	PTA	2PTA2	LID-1
HPMPC	11	20	13	12
cHPMPC	11	13	9.5	7.8
HPMPA	3.2	3.6	2.6	2.6
PMEA	4.0	2.9	2.9	2.5
PMEDAP	7.8	6.5	3.5	4.3
PMEG	5.9	2.7	2.8	2.2
ACV	<3.5	<3.5	<3.5	<3.5
GCV	<3.6	<3.6	<3.6	<3.6
BVDU	<3.7	<3.7	<3.7	<3.7
PFA	>1.7	>1.9	>1.4	>1.6
Ara-A	2.8	2.9	2.2	2.8
Ribavirin	<1.9	<1.9	<1.9	<1.9

selectivity indices for these compounds. Ara-A and PFA showed IC_{50}s in the range of 35 to 45 and 104 to 143 μg/ml, while their CC_{50}s were 99 and >200 μg/ml, respectively. From these data, it is apparent that these two compounds showed a low selectivity index. Neither ACV, GCV, BVDU, or ribavirin showed any activity against murine polyomavirus.

Inhibitory effects of various antiviral compounds on SV40. SV40 strain A2895, SV40 PML-1 strain EK, and SV40 PML-2 strain DAR were evaluated for their susceptibility to several drugs in BS-C-1 cell cultures. The IC_{50}s and the selectivity indices for the various compounds are presented in Tables 3 and 4, respectively.

As noted for the murine polyomavirus, HPMPC and cHPMPC also emerged as the most selective inhibitors of SV40. The IC_{50}s for HPMPC and cHPMPC were in the range of 3.8 to 5.4 and 6 to 11.4 μg/ml, respectively, and their selectivity indices varied from 23 to 33 and 17 to 27, respectively. HPMPA and PMEG showed IC_{50}s that were close to the cytotoxicity threshold, which means that their selectivity indices were in the range of 2.5 to 6.0 and 7.6 to 11, respectively. PMEA and PMEDAP had no effect on SV40-, SV40 PML-1-, and SV40 PML-2-induced cytopathicity in BS-C-1 cells, and neither did the other compounds included in this assay (ACV, GCV, BVDU, PFA, Ara-A, Ara-C, and ribavirin).

TABLE 3. Susceptibility of SV40 and SV40-like human viruses to selected antiviral compounds in BS-C-1 cells

Compound	CC_{50} (μg/ml)	IC_{50} (μg/ml) for:		
		SV40 strain A2895	SV40 PML-1 strain EK	SV40 PML-2 strain DAR
HPMPC	124 ± 9	3.8 ± 2.7	4.0 ± 3.4	5.4 ± 4.8
cHPMPC	160 ± 57	6.0 ± 3.7	7.1 ± 5.3	11.4 ± 9.8
HPMPA	15.5 ± 6.4	2.8 ± 1.6	2.6 ± 0.9	6.3 ± 3.3
PMEA	>200	>50	>50	>50
PMEDAP	>200	>50	>50	>50
PMEG	6.5 ± 6.5	0.77 ± 0.63	0.59 ± 0.34	0.85 ± 0.61
ACV	>200	>50	>50	>50
GCV	>200	>50	>50	>50
BVDU	>200	>50	>50	>50
PFA	>200	>200	>200	>200
Ara-A	161 ± 55	>50	>50	>50
Ara-C	0.7 ± 0.2	>0.5	>0.5	>0.5
Ribavirin	>200	>50	>50	>50

TABLE 4. Selectivity indices of antiviral compounds in BS-C-1 cells for SV40 and SV40-like human viruses

Compound	Selectivity index for:		
	SV40 (strain A2895)	SV40 PML-1 (strain EK)	SV40 PML-2 (strain DAR)
HPMPC	33	31	23
cHPMPC	27	23	14
HPMPA	5.5	6.0	2.5
PMEA	<4	<4	<4
PMEDAP	<4	<4	<4
PMEG	8.5	11.0	7.6
ACV	<4	<4	<4
GCV	<4	<4	<4
BVDU	<4	<4	<4
PFA	<1	<1	<1
Ara-A	<3.2	<3.2	<3.2
Ara-C	<1.4	<1.4	<1.4
Ribavirin	<4	<4	>4

Effect of various compounds on polyomavirus-induced CPE compared with their effect on cell proliferation in control and infected cell cultures. In order to verify the selectivity of HPMPC and cHPMPC in their antiviral effect against polyomaviruses, further experiments on cytotoxicity in infected and noninfected cell cultures tested in parallel with inhibition of virus-induced CPE were carried out. The results presented in Fig. 1 show that infection of BS-C-1 cells with SV40 PML-2 produced a marked decrease in the number of cells compared to control (noninfected) cell cultures. A close correlation between recuperation of cell number with inhibition of virus-induced CPE was observed in HPMPC- and cHPMPC-treated cultures. It should be noted that confluent control cultures continued to proliferate, and at concentrations of HPMPC and cHPMPC (5 to 20 μg/ml) at which marked inhibition of CPE was recorded, noninfected cells continued to proliferate while the number of cells in infected HPMPC- and cHPMPC-treated cultures increased compared to that in infected nontreated cultures. Neither ACV nor PMEA had any effect on virus-induced CPE or on the recuperation of the cell number in infected cultures.

Similar experiments were performed with PTA and LID-1 strains in UC1-B cells. However, infection with those viruses did not lead to cell death after 5 days (data not shown). Confluent control cultures continued to proliferate, and at virus-inhibitory concentrations of HPMPC and cHPMPC, infected and noninfected cells continued to proliferate (data not shown).

Effect of different times of addition on antipolyomavirus activity of HPMPC. To elucidate the stage of the polyomavirus replicative cycle affected by HPMPC, the compound was added at different times after viral infection. When HPMPC was added up to 10 h after viral infection, significant protection against the different murine polyomavirus strains was achieved (Fig. 2). When HPMPC was added later than 10 h after infection, its effect on viral cytopathicity began to decrease at different rates depending on the viral strain used. When HPMPC was added at 24 h postinfection, it had no effect on the replication of the LID-1 strain, while it had a significant inhibitory effect on the MN/RDE MN Toronto strain. Partial inhibition of PTA and 2PTA2 strains was noted when HPMPC was added at 24 h after infection. None of the murine polyomavirus strains was inhibited if HPMPC was added at 30 h postinfection.

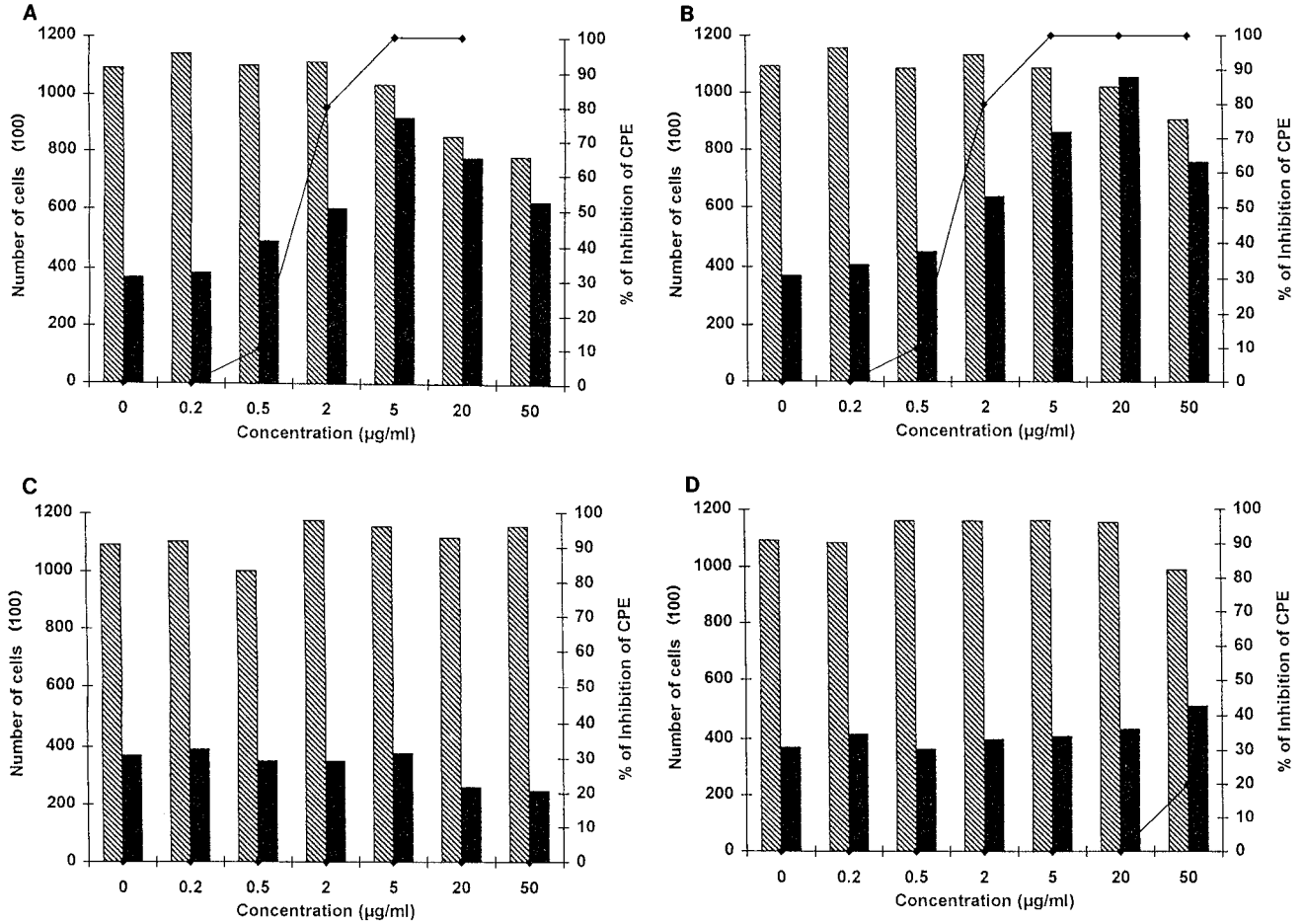


FIG. 1. Effect of HPMPC (A), cHPMPC (B), ACV (C), and PMEA (D) on SV40 PML-2-induced CPE and on cell proliferation in control (▨) and infected (■) cell cultures. Confluent monolayers of BS-C-1 cells were infected with SV40 PML-2, or left uninfected, and after a 2-h adsorption period, medium containing different concentrations of the compounds was added. After 7 days, viral CPE was monitored microscopically, and immediately thereafter, the number of cells in control (noninfected) and infected cell cultures was determined. The initial number of cells per culture was 719×10^2. ◆, percent inhibition of viral CPE.

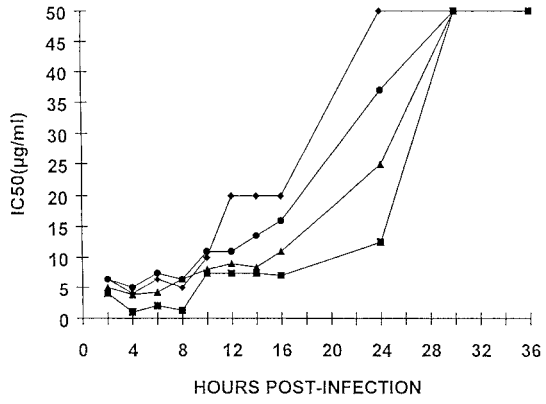


FIG. 2. Effect of the time of addition of HPMPC on mouse polyomavirus-induced cytopathicity. UC1-B cells were infected with the different mouse polyomavirus strains, and after a 2-h adsorption period, residual virus was removed, and the infected cells were further incubated with fresh medium. Medium containing different concentrations of HPMPC was added at various times postadsorption, and the cells were further incubated until viral cytopathicity was recorded after 4 to 5 days of infection. Symbols: ◆, LID-1; ■, MN/RDE Toronto; ▲, PTA; and ●, 2PTA2.

Significant inhibition of SV40 strains could be achieved even if the addition of HPMPC was delayed for up to 36 h after infection (Fig. 3). Thereafter, the inhibitory effect of HPMPC on viral cytopathicity began to decline.

Inhibitory effect of HPMPC on polyomavirus yield. To confirm that HPMPC resulted in significant inhibition of polyomavirus replication, virus yield assays were performed. HPMPC suppressed the growth of the LID-1 and PTA strains of murine polyomavirus in UC1-B cells in a concentration-dependent manner at nontoxic concentrations (Fig. 4A through D). Virus yield was decreased by 2 to 3 log units at an HPMPC concentration of 20 μg/ml. PMEA, PMEG, and HPMPA affected murine polyomavirus yield only at concentrations close to their CC_{50}s. Ribavirin, PMEDAP, and BVDU did not significantly affect the yield of polyomavirus. Also, a concentration-dependent inhibition of the SV40 strains was achieved by HPMPC at concentrations below its CC_{50}s for BS-C-1 cells (Fig. 5A through C). HPMPC reduced the growth of SV40, SV40 PML-1, and SV40 PML-2 by 2.5 to 3 log units at a concentration of 20 μg/ml. HPMPA and PMEG produced a similar reduction in virus yield but at concentrations of 20 and 2 μg/ml, respectively, which were close to their CC_{50}s for BS-C-1 cells (15 and 6.5 μg/ml for HPMPA and PMEG, respectively). Neither PMEA, PMEDAP, ribavirin, or BVDU had any effect on the yield of the SV40 strains.

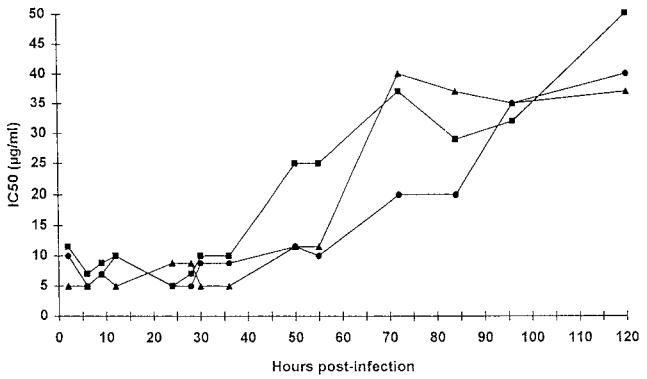


FIG. 3. Effect of the time of addition of HPMPC on SV40-induced cytopathicity. BS-C-1 cells were infected with the different SV40 strains, and after a 2-h adsorption period, residual virus was removed, and the infected cells were further incubated with fresh medium. Medium containing different concentrations of HPMPC was added at various times postadsorption, and the cells were further incubated until viral cytopathicity was recorded after 7 days of infection. Symbols: ●, SV40/PML-1; ■, SV40/PML-2; and ▲, SV40.

The effect of HPMPC on virus yield was also measured as a function of time. A concentration-dependent inhibition of SV40 PML-2 (Fig. 6A) and PTA (Fig. 6B) was observed at either 48, 72, or 96 h postinfection. Furthermore, for a given concentration of HPMPC, the difference in virus production compared with nontreated cultures was maintained or even increased as a function of time.

DISCUSSION

In the present study we describe the inhibitory effects of selected antiviral compounds on polyomavirus replication in vitro. Among the different compounds examined here, HPMPC and cHPMPC emerged as the most selective inhibitors of both murine polyomavirus and SV40 strains. The IC_{50}s for HPMPC were in the range of 4 to 7 μg/ml, and its selectivity index varied from 11 to 20 for murine polyomaviruses and from 23 to 33 for SV40 strains. Furthermore, it appeared that HPMPC and cHPMPC prevent cell death due to SV40 PML-2 replication (Fig. 1). A close correlation between recuperation of cell number and inhibition of virus-induced CPE was seen in HPMPC- and cHPMPC-treated cultures. Other acyclic nucleoside phosphonate analogs, i.e., HPMPA and PMEG, showed some activity against polyomavirus, but their selectivity was lower than that of HPMPC and cHPMPC. Also, PMEA and PMEDAP had only a slight inhibitory effect on the replication of murine polyomavirus and no effect on the replication of the SV40 strains. None of the other compounds included in this study (i.e., ACV, GCV, BVDU, PFA, Ara-A, Ara-C, and ribavirin) had a significant effect on polyomavirus replication. Although Ara-C has been reported to have some effect on the outcome of PML in patients (13, 20), Ara-C did not prove inhibitory to the replication of polyomaviruses at nontoxic concentrations (Table 3).

HPMPC shows potent activity against several DNA viruses, including herpesviruses, vaccinia virus, adenovirus, and papillomavirus (6, 7, 23, 25). Thus, according to our present findings, the broad-spectrum anti-DNA virus activity of HPMPC

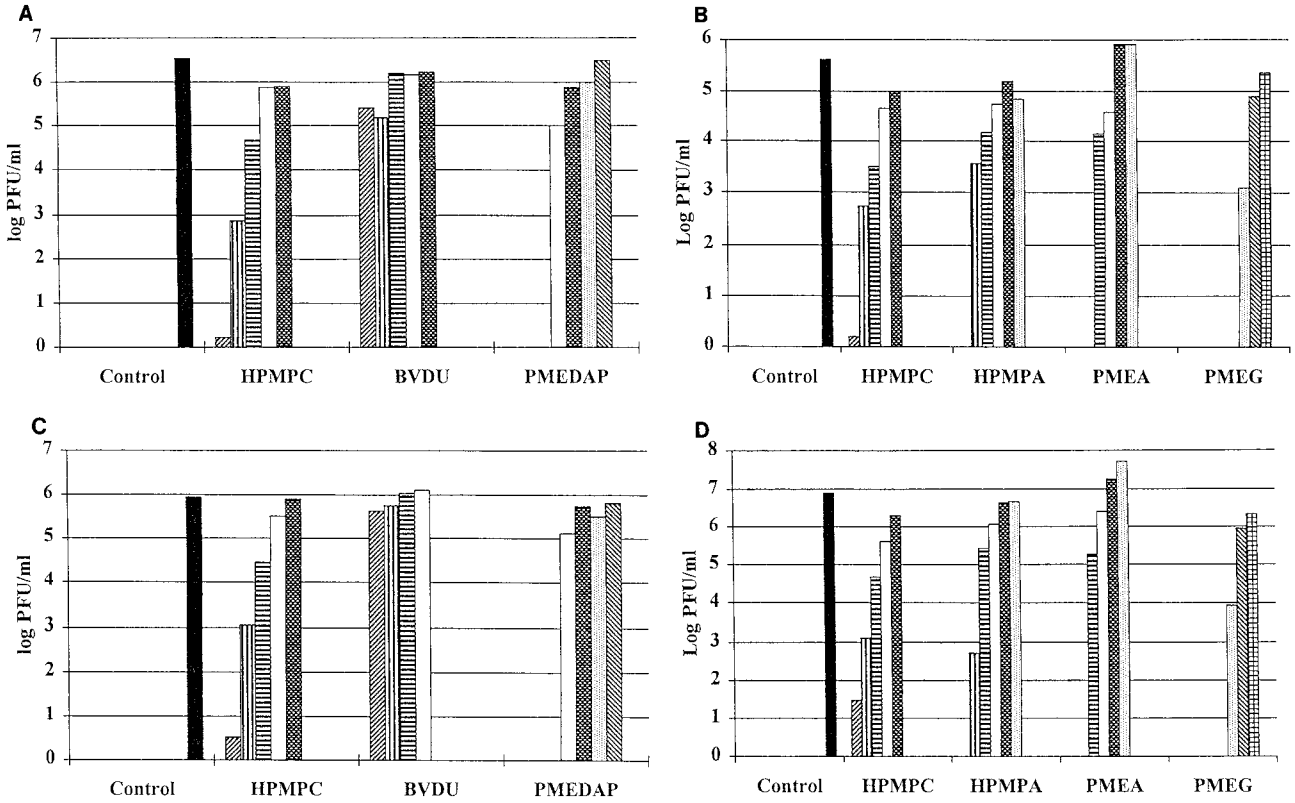
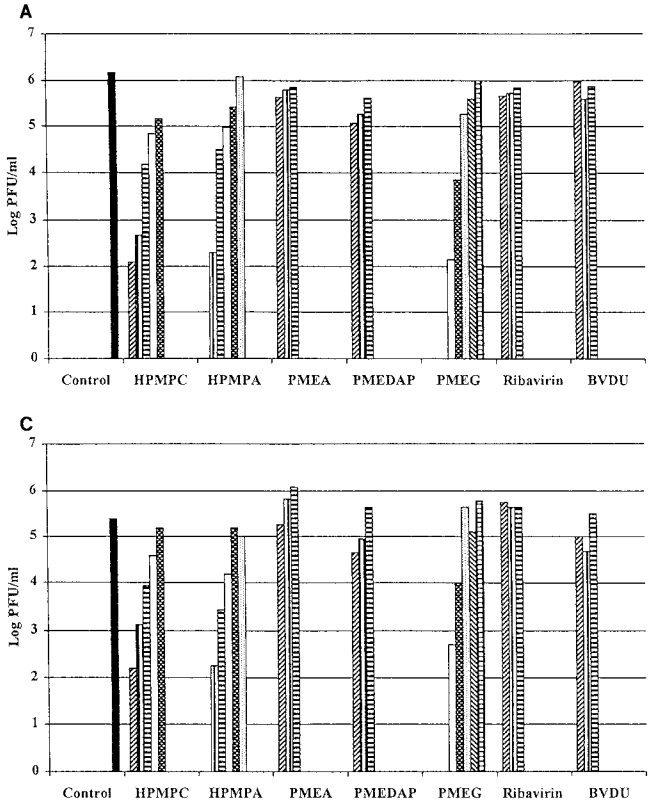


FIG. 4. Effect of different compounds on murine polyomavirus replication. Confluent monolayers of UC1-B cells were infected with the LID-1 strain (A and B) or PTA strain (C and D), and the various compounds were added at the end of virus adsorption. Virus titration was performed by plaque assay with UC1-B cells. Data represent the virus yield produced 6 days after infection in the presence or absence of various concentrations of the different compounds, as follows (in micrograms per milliliter): ▨, 50; ◫, 20; ⊟, 5; □, 2; ▩, 0.5; ▧, 0.2; ▨, 0.05; ⊞, 0.02; and ■, 0.



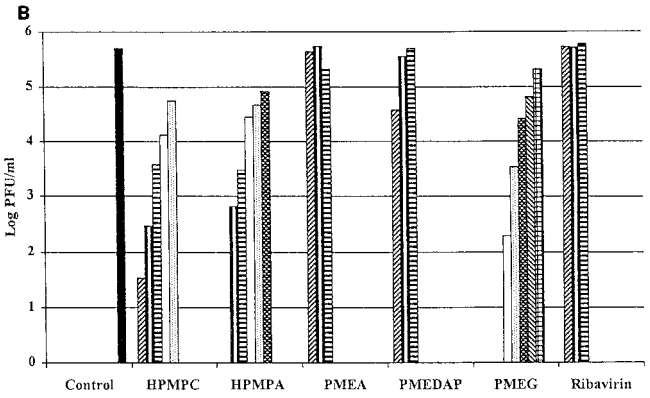


FIG. 5. Effect of different compounds on SV40 strain replication. Confluent monolayers of BS-C-1 cells were infected with SV40 (A), SV40 PML-1 (B), or SV40 PML-2 (C), and the various compounds were added at the end of virus adsorption. Virus titration was performed by plaque assay with BS-C-1 cells. Data represent the virus yield produced 4 days after infection in the presence or absence of various concentrations of the different compounds. Symbols are as defined for Fig. 4.

also extends to polyomavirus. The mechanism of action of HPMPC against herpesviruses (i.e., cytomegalovirus), where it inhibits viral DNA synthesis, has been mainly studied (14). HPMPC can be taken up by the cells and needs only two phosphorylations to be converted to the active diphosphoryl derivative (HPMPCpp). According to the inhibition constants of HPMPCpp for DNA polymerases from herpesviruses and human cells, HPMPCpp is a poor inhibitor of human DNA polymerases α, β, and γ, and concentrations that inhibit viral DNA polymerases are considerably lower than those that affect the human DNA polymerases. Thus, the antiviral selectivity of HPMPC can be ascribed to the higher affinity for viral than for human DNA polymerases. It has been shown that HPMPCpp acts as both an inhibitor and an alternative substrate for human cytomegalovirus DNA polymerase and reduces the rate of DNA synthesis (27). This inhibition is due to the fact that HPMPCpp acts as an alternative substrate in competition with dCTP.

The mechanism of action of HPMPC against polyomaviruses cannot be extrapolated from that described for herpesviruses, since polyomaviruses do not encode for their own DNA polymerase. The viral genome in polyomaviruses is approximately 5,000 bp and is divided into early and late regions, corresponding to the portions of the genome that are expressed during the early and late stages of infection (10). The early region encodes T antigens: SV40 and related viruses (JCV and BKV) encode two T antigens (small t and large T), while polyomavirus encodes three T antigens (small t, middle T, and large T). The late region of the viral genome encodes the three capsid proteins. The early stage of infection occurs prior to viral DNA replication and involves expression of genes

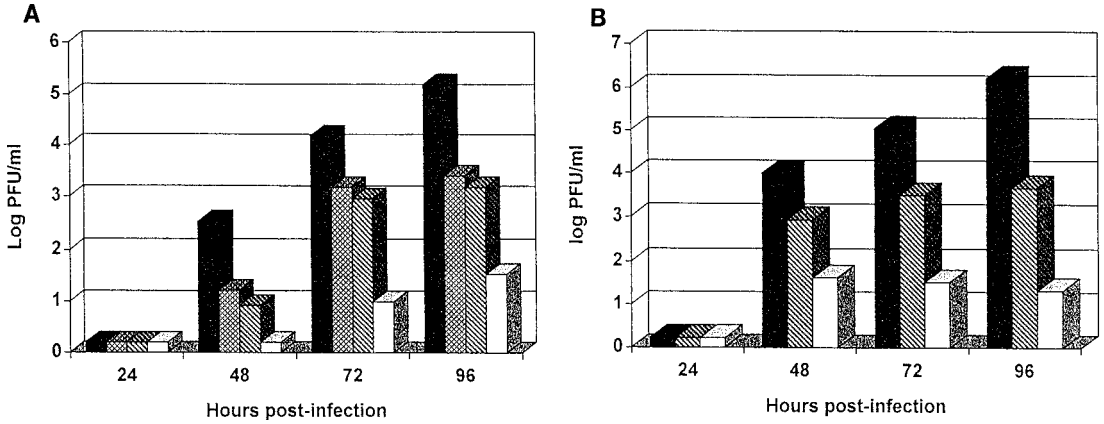


FIG. 6. Effect of HPMPC on SV40 PML-2 (A) or PTA (B) growth. Confluent cultures of BS-C-1 and UC1-B cells were infected with SV40 PML-2 or PTA, respectively. After a 2-h adsorption period, different concentrations of HPMPC were added. At different times postinfection, supernatants were harvested, and virus yield was determined by plaque assay in BS-C-1 or UC1-B cells for SV40 PML-2 and PTA strains, respectively. Symbols: ■, controls; ▩, 2 μg/ml; ▧, 5 μg/ml; □, 20 μg/ml.

leading to changes in the host cell, including activation of cellular gene expression and induction of cellular DNA synthesis. Shortly thereafter, viral DNA synthesis begins, followed by synthesis of virion proteins and assembly of progeny virus particles. A single infectious cycle by polyomavirus takes 24 to 48 h, while for SV40 a single cycle takes 48 to 72 h. According to the results obtained with time-of-addition experiments, it can be assumed that HPMPC does not affect the early stage of polyomavirus replication.

Although the replication forks in replicating viral DNA intermediates are similar to those in eukaryotic chromosomes, viral and cellular DNA may differ in the mechanism of initiating new rounds of replication, because cellular chromosomes replicate once per cycle whereas viral DNA replicates several times in a single cellular S phase. The large T antigen of polyomavirus and SV40 is required for initiation of viral DNA replication (8). T antigen binds to specific regions of viral DNA in the vicinity of the origin of replication. Both polyomavirus and SV40 T antigens have ATPase activities, while SV40 T antigen has adenylating activity and helicase activity that can unwind DNA molecules at the SV40 origin of replication. The large T antigen also binds to at least two cellular proteins involved in growth control, p53 and the retinoblastoma protein. Whether HPMPC affects one or more activities of the large T antigen that are involved in polyomavirus replication should be further explored.

Primary BKV and JCV infections occur during childhood and are generally asymptomatic. Seroepidemiologic studies have shown that 70 to 80% of adults have specific antibodies against the two viruses. The viruses persist indefinitely in a latent form, probably in the kidneys, although for JCV, lymphoid tissues such as bone marrow and spleen are considered sites for initial and/or latent infection. Immunologic impairments, especially conditions producing T-cell deficiencies, may lead to the reactivation of the virus and to virus excretion in the urine (18, 19). With the extensive use of immunosuppressive therapy in transplant recipients and patients with autoimmune diseases and malignancies and the increasing number of AIDS patients, a considerable increase in the occurrence of polyomavirus infections has been observed. In particular, a significant increase in the frequency of PML in HIV-infected patients has been noted (11, 22). The course of this disease is rapidly progressive and fatal, and there is currently no accepted treatment for polyomavirus infections. Thus, there is an urgent need for an effective therapeutic strategy. HPMPC has emerged as the most selective antipolyomavirus agent among all the compounds included in the present study of animal polyomaviruses and deserves further evaluation as a potential treatment for PML in AIDS patients.

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