Inhibitory Activities of Three Classes of Acyclic Nucleoside Phosphonates against Murine Polyomavirus and Primate Simian Virus 40 Strains

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Murine polyomavirus and simian virus 40 were used to evaluate the potencies of the compounds of three classes of acyclic nucleoside phosphonates: (i) the original HPMP (3-hydroxy-2-phosphonomethoxypropyl) and PME (2-phosphonomethoxyethyl) derivatives, (ii) the 6-[2-(phosphonomethoxy)alkoxy]-2,4-diaminopyrimidine (DAPy) derivatives, and (iii) a new class of HPMP derivatives containing a 5-azacytosine moiety. The last class showed the highest activities and selectivities against both polyomaviruses.

Polyomaviruses are widely distributed among vertebrates. Humans are the natural hosts for two viruses belonging to the Polyomaviridae family, i.e., JC virus (JCV) and BK virus (BK V), which can cause severe diseases in immunosuppressed patients. BKV and JCV are related to simian virus 40 (SV40), which was introduced into the human population through contaminated poliovirus and adenovirus vaccines during the 1950s and 1960s (6). Previously, various nucleoside analogs were evaluated for their activities against murine polyomavirus and SV40, which are easier to propagate in vitro than the human polyomaviruses (1). In that study, cidofovir ([S]-1-(3-hydroxy-2-phosphonomethoxypropyl)-cytosine (HPMPC); Vistide) emerged as the most selective compound. Currently, there is no approved specific antiviral treatment for JCV and BKV infections. One of the several treatment options that have been tried is based on the intravenous administration of HPMPC. This acyclic nucleoside phosphonate (ANP) proved to be effective in the treatment of BKV- and JCV-associated infections in immunocompromised patients (10, 14, 16, 20, 21, 22). However, some studies failed to show a beneficial effect of HPMPC in the management of these infections (11, 17). Therefore, there is an urgent need for development of new antipolyomavirus agents.

Recently, two new classes of ANPs, namely, 6-[2-(phosphonomethoxy)alkoxy]-2,4-diaminopyrimidine (DAPy) derivatives and HPMP derivatives with a 5-azacytosine moiety, have been developed. These compounds display antiviral activity spectra that are similar to those of the parent ANP compounds (2, 4, 8, 13). In the present study, SV40 and murine polyomaviruses were evaluated for their susceptibilities to the compounds of the three classes of ANPs. All compounds used are listed and

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### TABLE 1. ANPs used in this study

<table>
<thead>
<tr>
<th>Group</th>
<th>Designation(s) for compound</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>First class</td>
<td>HPMPC, CDV, cidofovir, Vistide</td>
<td>(S)-1-[3-Hydroxy-2-(phosphonomethoxy)propyl]cytosine</td>
</tr>
<tr>
<td></td>
<td>HPMPA</td>
<td>(S)-9-[3-Hydroxy-2-(phosphonomethoxy)propyl]adenine</td>
</tr>
<tr>
<td></td>
<td>HPMPDAP</td>
<td>(S)-9-[3-Hydroxy-2-(phosphonomethoxy)propyl]-2,6-diaminopurine</td>
</tr>
<tr>
<td></td>
<td>3-Deaza-HPMPA</td>
<td>(S)-9-[3-Hydroxy-2-(phosphonomethoxy)propyl]-3-deazaadenine</td>
</tr>
<tr>
<td></td>
<td>cHPMPA, cCDV, cyclic cidofovir</td>
<td>Cyclic (S)-HPMPC</td>
</tr>
<tr>
<td></td>
<td>CHPMPA</td>
<td>Cyclic (S)-HPMPC</td>
</tr>
<tr>
<td></td>
<td>PMEA, adefovir, Hepsera</td>
<td>9-[2-(Phosphonomethoxy)ethyl]adenine</td>
</tr>
<tr>
<td>Second class</td>
<td>HPMPO-DAPy</td>
<td>(R)-2,4-Diamino-3-hydroxy-2-[2-(phosphonomethoxy)propoxy]pyrimidine</td>
</tr>
<tr>
<td></td>
<td>PMEO-DAPy</td>
<td>2,4-Diamino-6-[2-(phosphonomethoxy)ethoxy]pyrimidine</td>
</tr>
<tr>
<td>Third class</td>
<td>HPMP-5-azaC</td>
<td>1-(S)-[3-Hydroxy-2-(phosphonomethoxy)propyl]-5-azacytosine</td>
</tr>
<tr>
<td></td>
<td>cHPMP-5-azaC</td>
<td>Cyclic HPMP-5-azacytosine</td>
</tr>
<tr>
<td></td>
<td>HDE-cHPMP-5-azaC</td>
<td>Hexadecyloxyethyl ester of cyclic HPMP-5-azacytosine</td>
</tr>
</tbody>
</table>

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defined in Table 1, and their structures are shown in Fig. 1. The murine polyomavirus strains, i.e., MN/RDE Toronto, PTA, 2PTA2, and LID-1 (ATCC VR-252), were grown in UC1-B cells (murine embryo fibroblasts, ATCC 6465-CRL). SV40 strain A2895 (ATCC VR-305), SV40 PML-1 strain EK (ATCC VR-820), and SV40 PML-2 strain DAR (ATCC VR-821) were propagated in BS-C-1 cells (African green monkey kidney cell line ATCC CCL-26). Cytopathic reduction assays were carried out as described previously (1). The viral cytopathic effect (CPE) was recorded, and the 50% effective concentration (EC50) was defined as the compound concentration required to reduce the viral CPE by 50% compared to that for the untreated control. The cell toxicities of the compounds were evaluated based upon inhibition of cell growth using a Coulter counter, and the evaluations were performed as reported before (1). The 50% cytotoxic concentration (CC50) was defined as the concentration required to reduce the number of cells by 50% compared to that for the untreated control. The selectivity index (SI) was calculated as the ratio of the CC50 for cell growth to the EC 50 for viral CPE.

The antiviral activities of the three classes of ANPs against murine polyomavirus and SV40 strains are shown in Table 2 and Table 3. The potencies of HPMPA, HPMPDAP, 3-deaza-HPMPA, and PMEA, all belonging to the first class of ANPs, against murine polyomavirus were similar to that of HPMPC (mean EC50 values against the four murine polyomavirus...
strains in the range of 7.7 μM to 12.6 μM, compared to 16.7 ± 2.8 μM for HPMPC). However, HPMA, HPMPDAP, 3-deaza-HPMPA, and PMEA showed poor selectivities (SIs of <5), while HPMPC proved to be selective, with a mean SI of 10. The cyclic counterparts of HPMPC and HPMA, i.e., cHPMPA and cHPMPC, proved to be less active than the parent compounds (mean EC₅₀ values of 48.1 ± 11.9 μM and 52.6 ± 11.7 μM, respectively, for cHPMPA and cHPMPC), with cHPMPC but not cHPMPA being a selective inhibitor of murine polyomavirus replication. Compounds belonging to the first class of ANPs inhibited the replication of SV40 strains in the following order of potency: 3-deaza-HPMPA > HPMPC > cHPMPC > HPMPO-DAPy > PMEA.

Similar to what was found for murine polyomaviruses, among this class of ANPs, only HPMPC and cHPMPC emerged as selective anti-SV40 compounds.

Two DAPy derivatives (i.e., HPMPO-DAPy and PMEO-DAPy) of the second class of ANPs were included in this study. The anti-murine polyomavirus activity of HPMPO-DAPy, with an average EC₅₀ of 11.1 ± 2.3 μM, was comparable to that of its alkyl purine counterpart (i.e., HPMPDAP) and to that of the reference compound HPMPC, whereas PMEO-DAPy (mean EC₅₀ of 14.7 ± 5.1 μM) proved to be as active as its PME counterpart. When the DAPy derivatives were tested against three SV40 strains, HPMPO-DAPy (average EC₅₀ of 86.1 ± 22.8 μM) was observed to be two- and fivefold less active than HPMPDAP and HPMPC, respectively. PMEO-DAPy (average EC₅₀ of 156.3 ± 21.9 μM) showed, similar to its alkylpurine counterpart, i.e., PMEA, a weak activity. For both murine polyomavirus and SV40, none of the DAPy compounds were selective.

Among the HPMP derivatives containing a 5-azacytosine moiety, HPMP-5-azaC and cHPMP-5-azaC exhibited antipolyomavirus potencies similar to those of HPMPC and cHPMPC, respectively. With average SIs of 23 and 28, respectively, for murine polyomaviruses and SV40 strains, HPMP-5-azaC

### Table 2. Activities and cytotoxicities of the tested compounds against different murine polyomavirus strains in UCI-B cells

<table>
<thead>
<tr>
<th>Compound</th>
<th>Activity (EC₅₀ [μM])a against:</th>
<th>CC₅₀ (μM)</th>
<th>SIb for:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MN/RDE</td>
<td>PTA</td>
<td>2PTA</td>
</tr>
<tr>
<td></td>
<td>Toronto</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HPMPDAP</td>
<td>0.04 ± 0.12</td>
<td>0.03 ± 0.07</td>
<td>0.08 ± 0.03</td>
</tr>
<tr>
<td>cHPMPA</td>
<td>0.05 ± 0.09</td>
<td>0.06 ± 0.08</td>
<td>0.08 ± 0.08</td>
</tr>
<tr>
<td>PMEO-DAPy</td>
<td>0.02 ± 0.02</td>
<td>0.08 ± 0.06</td>
<td>0.11 ± 0.07</td>
</tr>
<tr>
<td>cHPMP-5-azaC</td>
<td>0.01 ± 0.01</td>
<td>0.02 ± 0.02</td>
<td>0.03 ± 0.03</td>
</tr>
</tbody>
</table>

### Table 3. Activities and cytotoxicities of the tested compounds against different SV40 strains in BS-C-1 cells

<table>
<thead>
<tr>
<th>Compound</th>
<th>Activity (EC₅₀ [μM])a against indicated strain</th>
<th>CC₅₀ (μM)</th>
<th>SIb for indicated strain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SV40 A2895</td>
<td>SV40 PML-1</td>
<td>SV40 PML-2</td>
</tr>
<tr>
<td>HPMPDAP</td>
<td>0.02 ± 0.01</td>
<td>0.05 ± 0.06</td>
<td>0.06 ± 0.07</td>
</tr>
<tr>
<td>cHPMPA</td>
<td>0.01 ± 0.01</td>
<td>0.02 ± 0.02</td>
<td>0.03 ± 0.04</td>
</tr>
<tr>
<td>PMEO-DAPy</td>
<td>0.03 ± 0.04</td>
<td>0.05 ± 0.06</td>
<td>0.07 ± 0.08</td>
</tr>
<tr>
<td>cHPMP-5-azaC</td>
<td>0.02 ± 0.02</td>
<td>0.04 ± 0.04</td>
<td>0.05 ± 0.06</td>
</tr>
</tbody>
</table>

### Notes

a The EC₅₀ values for each compound represent the means ± standard deviations for the EC₅₀ values from at least three independent experiments. The CC₅₀ values represent the means ± standard deviations for the CC₅₀ values from at least two independent experiments.

b Concentration required to inhibit 50% of virus-induced CPE.

c Concentration required to reduce cell growth by 50%.

d SI: ratio of CC₅₀ to EC₅₀.
proved to be twofold more selective than HPMPC (for murine polyomaviruses) or as selective as HPMPC (for SV40). The prodrug form of cHPMP-5-azaC (HDE-cHPMP-5-azaC) clearly demonstrated the highest activities and selectivities of all compounds tested against murine (average EC$_{50}$ of 0.63 ± 0.17 µM and SI of 31) and primate (average EC$_{50}$ of 0.29 ± 0.06 µM and SI of 48) polyomaviruses.

The activities of HPMPC, cHPMPC, HPMP-5-azaC, cHPMP-5-azaC, and HDE-cHPMP-5-azaC against the LID-1, PTA, and SV40 A2895 strains were confirmed in a virus yield

FIG. 2. Virus yield reduction caused by HPMPC (molecular weight [MW], 315.12), cHPMPC (MW, 297.2), HPMP-5-azaC (MW, 280.18), cHPMP-5-azaC (MW, 262.16), and HDE-cHPMP-5-azaC (MW, 530.65) for the LID-1 (A) and PTA (B) strains in U1112-B cells and for the SV40 A2895 (C) strain in BS-C-1 cells. Symbols: ◼, HPMPC; □, cHPMPC; ▲, HPMP-5-azaC; ×, cHPMP-5-azaC; ●, HDE-cHPMP-5-azaC. The dotted line indicates the 2-log reduction (EC$_{90}$) compared to the level for the virus control.
reduction assay, carried out as previously described (1). As shown in Fig. 2, HDE-cHPMP-5-azaC (EC99 values of 0.81 \(\mu M\), 0.43 \(\mu M\), and 0.26 \(\mu M\) against the LID-1, PTA, and SV40 A2895 strains, respectively) emerged as the most potent inhibitor of both murine polyomavirus (Fig. 2A and B) and SV40 (Fig. 2C) replication. cHPMPc exhibited the least potent anti-LID-1 activity, with an EC99 of 111 \(\mu M\), followed by cHPMP-5-azaC (EC99, 84 \(\mu M\)), HPMPc (EC99, 44 \(\mu M\)), and HPMP-5-azaC (EC99, 18 \(\mu M\)). An identical order of activity could be seen in the virus yield reduction assay with PTA. The potencies of the compounds were similar to those for LID-1 (cHPMPc EC99, 64 \(\mu M\); cHPMP-5-azaC EC99, 42 \(\mu M\); HPMPc EC99, 16 \(\mu M\); and HPMP-5-azaC EC99, 14 \(\mu M\)). For SV40 strain A2895, cHPMP-5-azaC, with an EC99 of 8 \(\mu M\), proved to be more potent than HPMPc (EC99, 22 \(\mu M\)), cHPMPc (EC99, 30 \(\mu M\)), and HPMP-5-azaC (EC99, 43 \(\mu M\)) in the virus yield reduction assay.

As previously reported (1), we confirmed here that HPMPc and cHPMPc proved to be the most selective drugs of the first class of ANPs against both murine and primate polyomaviruses. They served as references for the evaluation of the two other classes of ANPs. Among them, the second class of ANPs (i.e., the DAPy derivatives), which has been shown to display antiviral activity spectra and potencies that closely resemble those of the parental HPMP- and PME-purine types of compounds (2, 4), also showed antipolyomavirus activities comparable to those of the parent compounds. However, none of the DAPy derivatives was selective against polyomaviruses. Recently, HPMP-5-azaC, the leading compound of the third class of ANPs, was shown to display antiviral activities against adenovirus, poxvirus, and herpesviruses comparable to, or even better than, those of the parent compound HPMPc (12, 13). In the present study, we demonstrated that HPMP-5-azaC and cHPMP-5-azaC have antipolyomavirus potencies similar to those of HPMPc and cHPMPc, respectively. In order to increase the cellular uptake and oral bioavailability of HPMPc, several alkoxalkyl esters have been designed. These compounds proved to be highly active and selective against adenovirus, poxvirus, human papillomavirus, cytomegalovirus, and herpes simplex virus (3, 7, 9, 15, 23). When these prodrugs were given orally to treat cowpox and vaccinia virus infections in mice, they were as active as HPMPc given parenterally (18). Randhawa et al. recently showed that compounds obtained by esterification of HPMPc with octadecacyloxyethyl, oleoyloxyethyl, and hexadecacyloxypropyl inhibited BKV replication in vitro up to 1,400-fold more than HPMPc (19). In the present study, the alkoxalkyl ester of cHPMP-5-azaC (HDE-cHPMP-5-azaC) was highly potent and selective against murine and primate polyomaviruses. This is in agreement with our observations for other DNA viruses (12).

In conclusion, we have demonstrated that among the compounds of the three classes of ANPs that were analyzed here for their anti-murine polyomavirus and anti-SV40 activities, the HPMP derivatives bearing the 5-azacytosine moiety showed the highest activities and selectivities. Further studies are needed to determine the clinical potential for these compounds to become new drug candidates for treatment of polyomavirus-infected patients. Previously, it was demonstrated that the mechanisms of action of HPMPc against herpes-, pox- and adenoviruses are based on the higher affinity of this compound for viral polymerases than for cellular DNA polymerases (5). Since polyomaviruses do not encode their own DNA polymerase, they depend on cellular DNA polymerases for their replication. Consequently, it is suggested that HPMPc might interfere with the function of viral and/or cellular proteins jointly involved in the replicative process of the virus. A potential target is the large T antigen, a viral oncoprotein that plays an essential role in viral DNA replication and modulates cellular signaling pathways (24). Further studies are currently under way to elucidate the mechanisms of action of ANPs against polyomaviruses, including the selection of resistant viruses under selective drug pressure.

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