Activities of Different Classes of Acyclic Nucleoside Phosphonates against BK Virus in Primary Human Renal Cells

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BK virus (BKV), a virus belonging to the polyomavirus family, is a circular double-stranded DNA virus that causes nephropathies in immunocompromised patients after kidney or bone marrow transplantation. The occurrence of polyomavirus-associated nephropathy in kidney transplant patients may trigger graft loss, and guidelines for the management of BKV infection have not yet been clearly established. Treatment of BKV nephropathy with cidofovir (CDV) ([S]-1-[3-hydroxy-2-(phosphonomethoxy)propyl]cytosine (HPMPC)), an acyclic phosphonate analogue of dCMP with a broad antiviral activity against DNA virus infections, has been proposed. The benefit of this small-molecule-based treatment has been evaluated only with a limited number of cases. In this study, we report the evaluation of three different classes of acyclic nucleoside phosphonates for their activities against BKV replication in two different primary renal cells: renal proximal tubular epithelial cells (RPTECs) and human renal cortical epithelial (HRCE) cells. The data indicate that besides HPMPC and its cyclic form, ([S]-1-[3-hydroxy-2-(phosphonomethoxy)propyl]-5-azacytosine (HPMP-5-azaC), cyclic HPMP (cHPMP)-5-azaC, hexadecyloxyethyl (HDE)-cHPMP-5-azaC, and 9-[2-(phosphonomethoxy)ethyl]guanine (PMEG) are the most selective inhibitors of BKV replication. On the contrary, leflunomide, which has also been proposed for the management of BKV-associated diseases, is not able to inhibit BKV replication at nontoxic concentrations.

BKV virulence, since specific tandem repeats in this region appear to act as enhancer elements of viral replication.

In healthy children, primary infection with BKV occurs asymptotically, and a seroprevalence rate ranged from 40% in 3-year-old children to 80% in 10-year-old children. In adults, epidemiological data confirm the high seroprevalence of BKV (70% to 90%) (24). After primary infection, BKV remains latent indefinitely in the kidney. In immunocompromised patients, reactivation of BKV can occur, especially after transplantation with concomitant immunosuppressant treatment. Two complications are observed: polyomavirus-associated nephropathy (PVAN) after kidney transplantation, which is the major cause of kidney graft loss, and hemorrhagic cystitis after bone marrow transplantation (40). In about 5% of renal transplant recipients, nephropathy due to a reactivation of BKV is observed, and in half of these cases, a loss of allograft function may occur (20, 35). Currently, there is no treatment approved by the Food and Drug Administration (FDA) for the management of polyomavirus infections, particularly for BKV-associated diseases. A reduction of the immunosuppressive treatment is the first option to manage BKV reactivation but with a significant risk of graft loss (19). The use of leflunomide, cidofovir (CDV), or quinolones has been proposed as an alternative, especially when a reduction of the immunosuppressive treatment cannot decrease BKV replication and prevent the development of hemorrhagic cystitis or PVAN (2, 6, 28). JCV-associated disease is generally seen in immunocompromised patients presenting progressive multifocal leukoencephalopathy (PML), a demyelinating disease of the brain. Despite the high seroprevalence of JCV in healthy adults (50%), JCV reactivates mainly in immunocompromised patients, and its replication takes place in oligodendrocytes, even if it can be...
detected in the urine of healthy subjects. The deterioration of the white matter usually leads to death, as there is no treatment for the management of JCV-associated diseases. PML has been discovered as a complication in a patient suffering from chronic lymphocytic leukemia and Hodgkin’s lymphoma (4). The cases of PML increased dramatically with the appearance of AIDS, but highly active antiretroviral therapy permitted a reduction in the incidence of PML in HIV-positive patients (8). A recent interest in patients suffering from autoimmune diseases (e.g., severe psoriasis, multiple sclerosis, and Crohn’s disease) and treated with natalizumab or efalizumab has emerged because PML was found to be a posttreatment complication (5, 17, 23, 26, 32, 43). Recently, some cases of immunocompetent patients presenting PML have been described (16, 33). Thus, there is an urgent need to discover new antipolyomavirus therapies, as these viruses are widely present in the human population and may lead to severe diseases.

(S)-1-[3-Hydroxy-2-(phosphonomethoxy)propyl]cytosine (HPMPC) (cidofovir [CDV]; Vistide) is a phosphonate analogue of dCMP that has shown antiviral activity against several DNA viruses both in vitro and in vivo (11, 41). The phosphonate moiety confers resistance to the cellular alkaline phosphatases and therefore increases the half-life of the drug (from 2.4 to 3.2 h). HPMPC monophosphate-choline, a “storage” form of this compound, permits the release of the drug over time and therefore increases its half-life. HPMPC has been approved by the FDA only for the treatment of cytomegalovirus (CMV) retinitis in AIDS patients. The activation of the drug occurs via two steps of phosphorylation catalyzed by the cellular UMP-CMP kinase and the nucleoside diphosphate kinase (7). The incorporation of two consecutive molecules of the active metabolite (i.e., HPMPC diphosphate) into the DNA by human CMV (HCMV) DNA polymerase has been shown to stop DNA elongation. The selectivity of HPMPC is based on the higher affinity of its active form for the viral DNA polymerase than for the cellular polymerases. The antiviral activity and mode of action of HPMPC against the Herpesviridae and Poxviridae was examined in detail previously (31, 47, 48). Previous studies have shown the antiviral activity of HPMPC against BKV and SV40 (3, 6, 25). Its mechanism of action is different from those described for herpesviruses and poxviruses because polyomaviruses do not encode their own DNA polymerase. For their replication, polyomaviruses are dependent on the host DNA polymerase. The LTag has a “helicase” activity that permits the unwinding of the viral genome at the “origin of replication” (ORI), and cellular proteins are recruited for the production of new viral DNA copies (42). The mechanism of action of HPMPC against polyomaviruses still has to be investigated.

New classes of nucleoside phosphonate derivatives have been developed with an aza group or a 6-[2-(phosphonomethoxy)alkoxy]-2,4-diaminopyrimidine (PMEO-DAPy) moiety on (S)-1-[3-hydroxy-2-(phosphonomethoxy)propyl] (HPMP) or 9-[2-(phosphonomethoxy)ethyl] (PME) scaffolds. These derivatives showed antiviral effects on a broad spectrum of DNA viruses and especially on SV40 and murine polyoma virus (mPyV) (27). In this study, we assayed the three different classes of phosphonate analogues (Fig. 1) for anti-BKV activities by the determination of their effects on the viral DNA.

FIG. 1. (A) Structures of the first class of ANPs. (B) Structures of the second and third classes of ANPs and non-ANPs.
load in two different primary human renal cell types, renal proximal tubule epithelial cells (RPTECs) and human renal cortical epithelial (HRCE) cells, infected with BKV.

MATERIALS AND METHODS

Cell cultures and viruses. Human embryonic lung (HEL) fibroblasts (ATCC CCL137) were maintained in minimum essential medium (MEM) supplemented with 10% inactivated fetal bovine serum (FBS), 1% t-glutamine, 1% sodium pyruvate, 1% HEPES, and 1% nonessential amino acids. HRCE cells and RPTECs (Lonzza) used for the antiviral and cytotoxic assays were maintained in renal epithelial cell growth medium (REGM) supplemented with 0.1% α-EGF (recombinant human epithelial growth factor), 0.1% insulin, 0.1% hydrocortisone, 0.1% GA-1000 (gentamicin and amphotericin B), 0.5% FBS, 0.1% epinephrine, 0.1% T3 (triothyronine), and 0.1% transferrin. Renal cells and supplemented medium were purchased from Lonza. Stocks of the human polyomavirus BKV (ATCC VR-837) were prepared in HEL fibroblasts and titrated in HRCE cells and RPTECs according to the method of Reed and Muench (36).

BKV growth curve. In order to establish the optimal time of incubation for the antiviral assay, BKV growth kinetic assay were performed during 10 days using both HRCE cells and RPTECs in supplemented REGM at 37°C and in 5% CO2. As no distinct or easily recognized cytotoxic effects (CPE) can be observed with BKV, the viral growth was quantified by the measurement of the viral DNA load using a quantitative real-time PCR (qPCR) method at different time points postinfection.

Antiviral assays. For antiviral assays, confluent RPTECs or HRCE cells grown in 96-well microtiter plates were infected with BKV at 100 TCID50 (1 TCID50 corresponds to the virus stock dilution that is infective for 50% of the cell cultures). After 2 h of incubation at 37°C in a 5% CO2 atmosphere, residual virus was removed and replaced by supplemented REGM containing serial dilutions of the test compounds (in duplicate). After 7 days of incubation, the entire monolayer of the infected cells (infected cells and supernatants) were frozen at −80°C until they were processed for DNA extraction and quantitative real-time PCR to determine the viral DNA load. The effective concentrations were calculated the EC50, EC90, and EC99 values for each compound represent the mean values of three independent experiments.

RESULTS

Inhibitory effects of the first class of ANPs on BKV. Kinetics of BKV growth in HRCE cells and RPTECs were determined in order to determine the optimal time to perform the antiviral assays (Fig. 2). In both cell types, a linear rate of replication, reaching the maximum level of the viral DNA load, was observed at 6 to 8 days postinfection, whereas a logarithmic phase of viral growth was noticeable between days 2 and 4 postinfection. We then decided to evaluate the effects of the compounds on the viral DNA load at 7 days postinfection. Three classes of acyclic nucleoside phosphonates (ANPs) were assayed with human renal cells (Table 1). The first class is composed of ANPs bearing a cytosine, a guanosine, an adenosine, or a daminopurine base (Fig. 1A). The second class groups the acyclic phosphonates with a diaminoimidazine base linked to a phosphonomethoxypropyl moiety at position C-6 (Fig. 1B). The third class includes cidofovir analogues bearing a modified base with a nitrogen atom instead of a carbon atom at position 5 (Fig. 1B). After 7 days of incubation in the presence of different compound concentrations, BKV DNA was extracted and purified, and qPCR was performed to quantify the BKV viral load in order to determine the EC50, EC90, and EC99 for each compound. Among the first class of ANPs assayed with HRCE cells, 9-[2-(phosphonomethoxy)ethyl]guanine (PMEG) and 9-[2-(phosphonomethoxy)ethyl]-N′-cyclopropyl-2,6-diaminopurine (cPMPEDAP) exhibited submicromolar EC50s of between 0.027 μM and 0.36 μM (Table 2). A second group of ANPs composed of 3-deaza-(5′)-3-hydroxy-2-(phosphonomethoxy) propyladenine (3-deaza-HPMPA), HPMPA, cyclic HPMPA (cHPMPC), HPMPA, and cyclic HPMPA (cHPMPA) showed EC50 in the range of 2.5 μM to 10 μM, while 9-[2-(phosphonomethoxy)ethyl]adenine (PMEA), 9-[2-(phosphonomethoxy)ethyl]-2,6-diaminopurine (cPMPEDAP) and (5′)-3-hydroxy-2-(phosphonomethoxy)propyl)-2,6-diaminopurine (HPMPDAP) had EC50 of between 13 μM and 64 μM, 3-deaza-HPMPA, HPMPA, cHPMPC, and PMEG showed selectivity indices of between 10 and 46 (Fig. 3 and Table 2). The best compound of this class of ANPs was PMEG, which was twice as selective as cyclic HPMPC. When assayed with RPTECs, the antiviral activities observed with this class of compounds were slightly similar to the ones obtained with HRCE cells, but the measured cytotoxicity was increased (Table 2). On the one hand, PMEG, cHPMPC, HPMPA, and cPMPEDAP proved to be selective compounds against BKV replication, with SIs of between 5.0 and 15. On the other hand, PMEDAP and 3-deaza-HPMPA, which exhibited selective antiviral effects when tested with HRCE cells, showed SI values below 3 in RPTECs.
Inhibitory effects of the second and third classes of ANPs and non-ANP compounds on BKV. PMEO-DAPy, (R)-{2,4-diamino-3-hydroxy-6-[2-(phosphonomethoxy)propyl]}pyrimidine (HPMPO-DAPy) as well as HPMP-5-azaC, chHPMP-5-azaC, and hexadecylxoyethyl (HDE)-HPMP-5-azaC belong to the second and third classes of ANPs, respectively (Fig. 1B). PMEO-DAPy and HPMPO-DAPy, the two diaminopyrimidine compounds, were very weak inhibitors of BKV replication in both HRCE cells and RPTECs (Table 2). Thus, the observed EC_{50}s were about 100 μM to 200 μM, and the selectivity indices were below 2 (Table 2), and furthermore, the measured CC_{50}s were found to be between 67 μM and 174 μM. Contrary to the second class of ANPs, the third class included more selective compounds, with EC_{50}s in the range of 0.0033 μM to 5.2 μM and SIs of between 11 and 1,900 (Fig. 3 and Table 2). The hexadecylxoyethyl ester prodrug of cyclic HPMP-5-azaC emerged as the most promising compound among the 3 series of ANPs tested with HRCE cells (Fig. 3 and Table 2). When the compounds were assayed with RPTECs, similar results were obtained, even if the cytotoxicity was higher than that for HRCE cells, leading to lower SIs (between 42 and 339) (Table 2).

Besides the ANPs, we also assayed vidarabine (araA), cytarabine (araC), and leflunomide. These non-ANP compounds exhibited poor antiviral activities, with EC_{50}s of between 250 μM and 483 μM in HRCE cells (Table 2).
The need to discover new antiviral compounds against polyomavirus infections is urgent, as there is no established antiviral therapy for the management of polyomavirus-related diseases. In a previous study, we investigated the in vitro antiviral activities of three classes of ANPs against primate SV40 and murine polyomavirus (mPyV) in BS-C-1 and UC1-B cells, associated diseases (Fig. 3).

### DISCUSSION

The EC₉₀ of araA and leflunomide, with pronounced cytotoxicities for both compounds were in the range of 364 μM to 535 μM, leading to extremely low SIs (0.02 to 0.12) (Table 2). araC showed a submicromolar EC₉₀ (0.86 μM) and a nanomolar cytotoxicity (0.040 μM), which leads to a nonselective compound. The antiviral profiles were similar with RPTECs: high EC₉₀ for araA and leflunomide, with pronounced cytotoxicity, and a low EC₉₀ and high cytotoxicity for araC (Table 2B). Based on these data, none of these three compounds can be considered promising drugs for the management of BKV-associated diseases (Fig. 3).

<table>
<thead>
<tr>
<th>Cell type and molecule</th>
<th>Mean EC₉₀ (μM) ± SD</th>
<th>Mean EC₉₀ (μM) ± SD</th>
<th>Mean EC₉₀ (μM) ± SD</th>
<th>Mean CC₅₀ (μM) ± SD</th>
<th>SI</th>
</tr>
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<tr>
<td>HCRE cells</td>
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<tr>
<td>HPMPC</td>
<td>8.74 ± 2.5</td>
<td>53 ± 14</td>
<td>243 ± 97</td>
<td>127 ± 10</td>
<td>14.5</td>
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<td>HPMA</td>
<td>6.04 ± 2.64</td>
<td>49 ± 7.7</td>
<td>115 ± 17</td>
<td>23 ± 9.7</td>
<td>3.8</td>
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<tr>
<td>HPMPDAP</td>
<td>26 ± 12</td>
<td>91 ± 25</td>
<td>156 ± 8.8</td>
<td>57 ± 4</td>
<td>2.1</td>
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<td>3-Deaza-HPMA</td>
<td>2.49 ± 0.66</td>
<td>31 ± 13</td>
<td>140 ± 15</td>
<td>25 ± 3</td>
<td>10</td>
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<tr>
<td>cHPMPC</td>
<td>5.32 ± 0.77</td>
<td>39 ± 5.3</td>
<td>175 ± 24</td>
<td>150 ± 8</td>
<td>28</td>
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<td>cHPMPA</td>
<td>10.2 ± 3.1</td>
<td>43 ± 3.2</td>
<td>118 ± 9</td>
<td>16.5 ± 0.8</td>
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<td>PMEA</td>
<td>64 ± 15</td>
<td>680 ± 67</td>
<td>&gt;750</td>
<td>143 ± 5</td>
<td>2.2</td>
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<td>PMEDAP</td>
<td>13 ± 4.5</td>
<td>266 ± 14</td>
<td>&gt;700</td>
<td>100 ± 42</td>
<td>7.7</td>
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<td>cPrPMEDAP</td>
<td>0.360 ± 0.003</td>
<td>2.07 ± 0.76</td>
<td>12.2 ± 2.7</td>
<td>1.58 ± 0.73</td>
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<td>PMEG</td>
<td>0.027 ± 0.014</td>
<td>0.35 ± 0.31</td>
<td>3.35 ± 2.56</td>
<td>1.24 ± 0.30</td>
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<td>HPMO-DAPy</td>
<td>98 ± 97</td>
<td>468 ± 114</td>
<td>&gt;680</td>
<td>122 ± 14</td>
<td>1.2</td>
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<td>PMO-DAPy</td>
<td>139 ± 42</td>
<td>&gt;757</td>
<td>&gt;757</td>
<td>200 ± 37</td>
<td>1.4</td>
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<td>HPM-5-azaC</td>
<td>5.25 ± 2.2</td>
<td>45 ± 12</td>
<td>325 ± 222</td>
<td>57 ± 4</td>
<td>11</td>
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<td>cHPMP-5-azaC</td>
<td>2.29 ± 1.33</td>
<td>26 ± 13</td>
<td>144 ± 35</td>
<td>733 ± 95</td>
<td>320</td>
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<td>HDE-cHPMP-5-azaC</td>
<td>0.0033 ± 0.0015</td>
<td>0.047 ± 0.004</td>
<td>0.49 ± 0.13</td>
<td>6.30 ± 3.2</td>
<td>1909</td>
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<td>Leflunomide</td>
<td>364 ± 47</td>
<td>657 ± 30</td>
<td>740 ± 65</td>
<td>111 ± 10</td>
<td>0.3</td>
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<tr>
<td>araA</td>
<td>535 ± 56</td>
<td>&gt;750</td>
<td>&gt;750</td>
<td>9.36 ± 4.87</td>
<td>0.02</td>
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<tr>
<td>araC</td>
<td>0.86 ± 0.41</td>
<td>4.2 ± 1.0</td>
<td>10 ± 2.8</td>
<td>0.040 ± 0.006</td>
<td>0.05</td>
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</table>

*The EC₉₀ (concentration required to reduce the viral DNA load by 50%) values for each compound represent the means ± standard deviations for the EC₉₀ values from at least three independent experiments. The CC₅₀ (concentration required to reduce cell growth by 50%) values represent the means ± standard deviations for the CC₅₀ values from at least three independent experiments. SI, CC₅₀/EC₉₀ ratio.*
respectively (27). Among the first class of ANPs, HPMPC and cHPMP was found to be potent and selective anti-SV40 compounds, with SI values of 20 and 40, which is consistent with the data that we obtained for BKV. The other ANPs of the first class were less potent antiviral analogues against SV40 and mPyV, but the SI values were better than the ones obtained with BKV. The anti-mPyV activity was less pronounced for the different strains assayed. Among the second class of ANPs, neither HPMP-DAPy nor PMEO-DAPy could be considered an effective antipolyomavirus compound when assayed against SV40, mPyV, or BKV. The 5-aza derivatives of HPMPC were found to be more potent antiviral compounds against BKV than against SV40 or mPyV. The selectivity of HDE-HPMP-5-azaC for BKV was 40-fold higher than that for SV40 or mPyV, due mainly to a lower EC50 against BKV (27).

Since BKV does not induce a clear cytopathic effect in renal cells, the quantification of the viral DNA load by qPCR appears to be a more sensitive and convenient method for the determination of the antiviral activities of compounds. In the present study, we have measured the effects of different drugs on both intracellular and extracellular virus produced at 7 days postinfection. One limitation of this study is the fact that viral replication was measured as the viral DNA load and not as infectious virus produced or cytopathic effects due to the fact that BKV does not induce a clear CPE in infected cells. However, the antiviral effects of cidofovir on BKV and JCV have been previously reported by Pear et al. (27). Among the first class of ANPs, HPMPC and leflunomide (in combination with a reduction of immunosuppression) against BK virus, but others failed to demonstrate any benefit for the prevention of graft loss (22). Kuyers et al. showed previously that among eight patients with PVAN treated with CDV after a reduction of immunosuppression, none of them underwent graft loss. On the contrary, graft loss was observed for 70% of the cases of the control group (25). Leflunomide is known to be an inhibitor of the cellular dihydroorotate dihydrogenase that cause a reduction in the production of UMP and an increased expression of the tumor suppressor protein p53. Thus, leflunomide targets a host protein and has an effect on the metabolism of the infected cells, which may explain the antiviral effect reported by previous studies (46). In our system, leflunomide, whose active form is teriflunomide, exhibited a poor antiviral activity and a high toxicity, leading to a weak selectivity index. According to these data, we cannot consider leflunomide as a specific antiviral agent for the treatment of polyomavirus infections.

HPMP-5-azaC and its HDE derivative emerged as the most promising nucleotide analogues among the three classes of ANPs assayed. They were by far the most selective anti-BKV ANPs in HRCE cells, with SI values of 320 and 1,909, respectively. In contrast to leflunomide, cHPMP-5-azaC and HDE-cHPMP-5-azaC were less cytotoxic in both cell types and therefore more selective. If these drugs should be used in combination with a reduction in the immunosuppressive treatment, their relatively low toxicity could be an advantage. EC50s measured for HPMP-5-azaC and its prodrug were significantly lower than the CC50 values for both cell types. However, if these cidofovir analogues exhibit nephrotoxicity, an additional treatment could be the use of oral probenecid (4-g course), which may facilitate the clearance of the active metabolite, as it helps the elimination of cidofovir (at 5 mg/kg of body weight or greater), leading to an elimination half-life ($t_{1/2}$) of 2.5 h and a reduced volume of distribution at steady state ($V_{ss}$).

Its protective effect on the kidneys was demonstrated previously by Cundy et al. (9).

Interestingly, PMEG showed good results in HRCE cells and RPTECs, with a selectivity of the same range as that seen for HPMP-5-azaC. In previous studies, PMEG showed antiproliferative activity in vitro against human leukemic cells and solid tumor cell lines (34, 37). Rose and colleagues previously demonstrated the in vivo antiproliferative effect of PMEG on 2 types of mouse transplantable tumors (39). The development of PMEG as an antiviral drug was not considered due to its cytotoxicity. However, in the particular case of BKV, the EC50 was low enough to achieve promising selectivity indices for both types of renal cells. Furthermore, investigations of the antiviral effects of PMEG on BKV should be performed in vivo. PMEDAP, which bears an amino group at position 6 instead of an oxygen atom as in PMEG, was not a selective anti-BKV compound. Recently, Wolfgang et al. showed the antiproliferative effect of PMEG and its produgs cPrPMEDAP and GS-9191 (an ester prodrug of PMEG) on human papillomavirus-transformed cell lines (45). PMEG diphosphate was shown to inhibit cellular DNA polymerases α and β, preventing the replication of the cellular genome. To be selective as an antipolyomavirus compound, PMEG should have a higher affinity for one of the viral proteins than for cellular DNA polymerase α or β. The EC50s and CC50s measured in this work for both cell types are consistent with this hypothesis.

The mechanism of action of HPMPC against polyomaviruses has not yet been elucidated, but it is admitted that the target is not a viral DNA polymerase, like in herpesviruses and poxviruses, because polyomaviruses do not encode their own DNA polymerase, and they use cellular DNA polymerases for the replication of their genome. This host cell DNA polymerase-dependent mechanism of polyomavirus replication may imply the existence of another target for the ANPs. Since the large and the small T antigens are both involved in viral replication, they may be considered possible targets for ANPs. The unwinding of the viral circular DNA of polyomaviruses by the ATP-dependent helicase activity of the large T antigen could be considered a critical step for the inhibition of viral replication. However, a cellular DNA polymerase-dependent mechanism cannot be excluded, because in BKV-infected cells, the cell cycle is stimulated to increase viral replication, which could trigger more sensitivity to the drug. The expression of the T antigen is required for the replication of BKV but also for the maintenance of MCV-positive Merkel cell carcinoma, one of the most aggressive skin cancers (21). Furthermore, the management of JCV-related progressive multifocal leukoencephalopathy with HPMPC was reported previously and showed a favorable outcome (13, 33, 38). However, a multicenter observational study failed to demonstrate the influence of cidofovir treatment on PML-related mortality (12). The efficiency of HPMPC treatment in JCV-associated disease could be correlated to the immune status of the patient, as neurologic improvements have been observed for immunocompetent patients (33).
trichodysplasia spinulosa, a rare skin disease in immunocompromised patients (e.g., transplant recipients), has been associated with a newly discovered polyomavirus (TSPyV). Thus, seven members of the family Polyomaviridae are able to infect humans.

Treatment with topical 1% cidovifor cream twice a day resulted in a resolution of the lesions and a reduction of the viral load in patients suffering from trichodysplasia spinulosa (44). Thus, a growing body of experimental evidence suggests the need for new antiviral compounds with antipolyomavirus activity and, in some specific cases, the possible success of a small-molecule-based strategy to treat polyomavirus-associated diseases.

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