Anti-BK Virus Activity of Nucleoside Analogs.

Parmjeet Randhawa¹, Jiri Zemlicka², Andreas Sauerbrei³, Chris Meier⁴, Karl Y. Hostetler⁵, James R. Beadle⁶, Noush Afarin Farasati¹, Yuchen Huang¹, Matthews Bradley⁵

Division of Transplantation Pathology, Department of Pathology, University of Pittsburgh Medical Center¹
Developmental Therapeutics Program, Barbara Ann Karmanos Institute, Wayne State University School of Medicine²
Wayne State University School of Medicine, Detroit, Michigan 48201-1379²
Institute of Virology and Antiviral Chemotherapy, University of Jena, Jena, Germany³
Institute of Organic Chemistry, University of Hamburg, Hamburg, Germany⁴
Dept. of Medicine, University of California, San Diego, La Jolla, CA 92093-0676⁵
Luitpold Pharmaceuticals, Annapolis, MD⁶

This work was supported by NIH grants, AI51227¹, AI63360¹, AI66499⁵ and CA32779² and NIAID Contract # N01-AI-30044¹.

Running title: Anti-BK viral drugs
Keywords: BK virus, nucleoside, PMEA, (S)-HPMPA, methylenecyclopropanes, HDP-(S)-HPMPA.

Abstract: 50 words
Manuscript: 930 words

*Address correspondence to:
Parmjeet Randhawa, M.D.,
Professor, Division of Transplantation Pathology,
Department of Pathology, University of Pittsburgh,
Room E737 UPMC-Montefiore Hospital,
3459 Fifth Ave, Pittsburgh, PA 15213.
Phone: 412 647 7646
Fax: 412 647 5237
randhawapa@upmc.edu
ABSTRACT

Polyomavirus BK is an important pathogen in transplant recipients with no effective therapy. This study demonstrates that alkoxyalkyl esters of (S)-9-(3-hydroxy-2-phosphonylmethoxypropyl) adenine and fatty acid derivatives of 9-[2-(phosphonomethoxy)ethyl]adenine (viraprexins, P393 and P405), are potent and selective inhibitors of BKV replication, in vitro, with EC50 in the micromolar to nanomolar range.
Polyomaviruses are widely latent DNA viruses, of which the most important species is BK virus (BKV). In renal transplant recipients, BKV is reactivated in 20-60% of subjects, and nephropathy develops in up to 10%. BKV is also associated with hemorrhagic cystitis in up to 60% of bone marrow transplant patients (19, 26). No effective antiviral therapies are currently available. Although some medical centers have empirically used leflunomide and cidofovir, no proven clinical benefit resulted (10, 23, 25).

We investigated the antiviral activity of several nucleoside analogs using BKV Gardner strain (ATCC # VR837) grown in log phase WI-38 cells (ATCC# CCL-75) (7), in a 7 day quantitative PCR assay for viral replication. Toxicity was evaluated by the conventional neutral red assay, and by quantifying the housekeeping gene aspartoacylase. Technical details of these methods have been published (6, 19, 20). Selected chemical structures are depicted in Figures 1 and 2, and the results of testing are summarized in Table 1.

Acyclic nucleoside phosphonates were tested because this class of compounds encompasses several clinically useful antiviral agents. In our system, 9-[[2-( phosphonomethoxy)ethyl]adenine (PMEA) showed no significant activity (SI=1.34), confirming and extending prior work done with mouse polyomavirus (1). The prodrug form PMEA dipivoxil was approximately 2 logs more potent, but showed only a marginal increase in selectivity (SI= 5.74). However, fatty acid derivatives of PMEA, namely viraprexin P393 and P405 (Figure 1), showed striking activity. P393 exhibited CC50, EC50, and SI of respectively 165.3 ±11.6 µM, 1.0±0.31 µM, and 158.9 respectively. P405 had a comparable EC50
(2.27±0.03 µM), but the CC50 was > 100 µM and >200 µM in the first two experiments. By repeating the experiment in the concentration range of 100-1000 µM, a more precise value of 528.6 µM, was obtained and this generated a SI of 232.8. The mechanism by which fatty acid side chains enhance the efficacy of the parent compound was not determined. The possibility of increased transport into the infected cells was considered, but one might have expected this to have resulted in lowering of the CC50, and this was not observed. Notably, while PMEA dipivoxil also increased cell permeability compared to the parent compound, as reflected by a lower EC50, it did not have the same selectivity as the viraprexins.

(S)-9-(3-hydroxy-2-phosphonylmethoxypropyl)-adenine ((S)-HPMPA) is the adenine analog of the broad spectrum compound cidofovir (HPMPC). (S)-HPMPA is a broad spectrum antiviral agent with demonstrated activity against orthopox viruses, cytomegalovirus, herpesvirus 6, adenovirus, and hepatitis B virus(2, 4, 9, 18, 21, 24). We have previously reported that the hexadecyloxypropyl ester of cidofovir (HDP-CDV) is very active against BK virus in vitro (19). Our current work shows that the hexadecyloxypropyl (HDP) ester of (S)-HPMPA is the most active compound tested to date with CC50, EC50, and SI values of 0.8±0.4 µM, 0.015±0.006 µM, and 58.5. HDP-(S)-HPMPA is roughly 9 times more active in vitro against BKV than HDP-CDV (19). The parent compound (S)-HPMPA was recently reported not to be active against multiple strains of mouse and monkey polyomavirus (11). In the latter study, HPMP derivatives containing a 5-azacytosine moiety were shown to have the best
activity: the highest selectivity index (58.3) was found for the compound hexadecyloxyethyl-cHPMP-5-azaC, with other related compounds showing SI < 30.0.

There is recent interest in profiling the antiviral activity of methylenecyclopropane analogs of nucleosides (Figure 2)(27). The rationale is to introduce methylene groups, reduce the number of rotatable bonds, and increase the entropy factor, thereby, altering the biologic properties of the compounds (28). Modification of acyclovir and ganciclovir based on this principle has been used to generate a new class of anti-herpesvirus compounds. However, in our assay cyclopropavir, synguanol, synadenol, and ZSM-I-32-F showed no significant anti-viral activity.

Nucleoside analogs that inhibit the human immunodeficiency virus enzyme reverse transcriptase were also tested (5). Several of these compounds are active against hepatitis B, a DNA virus with a life cycle that includes an RNA intermediate that is reverse transcribed back to the DNA genome (8, 12). We evaluated the anti-BKV activity of these compounds, which are already approved by the Food and Drug Administration (FDA). Didanosine, lamivudine, and stavudine, were all found to be inactive at the screening concentration of 100µM. Famciclovir and tenofovir disoproxil also showed no effect at concentration limits imposed by the solubility of these compounds. Zidovudine (azidothymidine) was inactive.

The nucleoside analogs acyclovir and brivudine have previously been tested against several polyomavirus strains and found to have a very low
selective index (1). However, the synthesis of cycloSal-nucleoside monophosphates results in compounds that have greatly increased activity against orthopoxviruses, herpesviruses and HIV (3, 13-16, 22). Hence, we tested cycloSal-nucleoside monophosphates of acyclovir and brivudine (5-H-cycloSal-acyclovir-monophosphate and 3-methyl-cycloSal-3’-OH-BVDU-monophosphate) in our system, but found no significant antiviral activity (Table 1). The failure of the cycloSal strategy for BK virus is likely related to the fact that the small 5kb genome encodes neither a thymidine kinase nor a DNA polymerase. The presumed increased intracellular uptake of cycloSal compounds with subsequent release of the nucleoside by chemical hydrolysis, therefore, did not translate into reduced BKV replication.

In conclusion, we tested several compounds for anti-BKV activity in vitro. The most active compound was HDP-(S)-HPMPA which had an EC$_{50}$ of 0.015 $\mu$M and a selective index of 58. The viraprexins, P393 and P403, were less active with EC$_{50}$ values of 1.0 to 2.27 $\mu$M and selective indexes of 158 and 232 respectively. The other compounds lacked either efficacy or selectivity against BKV in vitro.
REFERENCES


TABLE 1: Anti-BK Virus Activity of Selected Nucleoside Analogs

<table>
<thead>
<tr>
<th></th>
<th>CC50 (µM)</th>
<th>EC50 (µM)</th>
<th>SI (CC50/EC50)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ACYCLIC NUCLEOSIDE</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PHOSPHONATES</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PMEA</td>
<td>124.7+/−37.8</td>
<td>95.8+/−18.2</td>
<td>1.3</td>
</tr>
<tr>
<td>PMEA dipivoxil</td>
<td>3.1+/−0.7</td>
<td>0.7+/−0.3</td>
<td>5.7</td>
</tr>
<tr>
<td>PMEA derivative P393</td>
<td>165.3+/−11.6</td>
<td>1.0+/−0.3</td>
<td>158.9</td>
</tr>
<tr>
<td>PMEA derivative P405</td>
<td>528.6</td>
<td>2.3+/−0.03</td>
<td>232.8</td>
</tr>
<tr>
<td>HDP-(S)-HPMPA</td>
<td>0.8+/−0.4</td>
<td>0.02+/−0.006</td>
<td>58.5</td>
</tr>
<tr>
<td>ODE-(S)-HPMPA</td>
<td>0.5+/−0.2</td>
<td>0.03+/−0.01</td>
<td>11.7</td>
</tr>
<tr>
<td><strong>CYCLOSAL DERIVATIVES</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-H-cycloSal-acyclovir-monophosphate</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>1.0</td>
</tr>
<tr>
<td>3-methyl-cycloSal-3′-OH-BVDU-monophosphate</td>
<td>98.3</td>
<td>41.9</td>
<td>2.3</td>
</tr>
<tr>
<td><strong>CYCLOPROPANE DERIVATIVES</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyclopropavir</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>1.0</td>
</tr>
<tr>
<td>Synadenol</td>
<td>259.6+/−65.5</td>
<td>44.2+/−0.9</td>
<td>5.9</td>
</tr>
<tr>
<td>Synguanol</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>1.0</td>
</tr>
<tr>
<td>ZSM-I-32-F</td>
<td>312+/−82</td>
<td>40.8+/−14.4</td>
<td>11.9</td>
</tr>
<tr>
<td><strong>NUCLEOSIDE RT INHIBITORS</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Didanosine</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>1.0</td>
</tr>
<tr>
<td>Compound</td>
<td>EC50 (µM)</td>
<td>SI</td>
<td>Mean</td>
</tr>
<tr>
<td>-------------------------</td>
<td>-----------</td>
<td>----</td>
<td>------</td>
</tr>
<tr>
<td>Famciclovir</td>
<td>&gt;0.78</td>
<td>&gt;0.78</td>
<td>1.0</td>
</tr>
<tr>
<td>Stavudine</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>1.0</td>
</tr>
<tr>
<td>Lamivudine</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>1.0</td>
</tr>
<tr>
<td>Tenofovir disoproxil</td>
<td>&gt;50</td>
<td>&gt;50</td>
<td>1.0</td>
</tr>
<tr>
<td>Azidothymidine</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>1.0</td>
</tr>
</tbody>
</table>

1. See text for compound abbreviations. PMEA derivatives P393 and P405 were contributed by Dr Bradley, HPMPA derivatives by Dr Hostetler, cyclosal derivatives by Dr Sauerbrei, and cyclopropane derivatives by Dr. Zimlicka. All other compounds were purchased from Sigma Chemicals, St Louis, Missouri, or from the pharmacy at the University of Pittsburgh Medical Center.

2. EC50 and SI data presented in this table was calculated by the neutral red assay. All results expressed as mean +/- sd are based on at least 3 experiments, except PMEA (tested twice).
Figure Legends:

Figure 1: Chemical structure of viraprexins P393 and P405.
Figure 2: Methylene cyclopropane Analogs of Nucleoside Analogs Tested for Anti-BKV Activity.
Figure 1
Figure 2