BK VIRUS REPLICATION IN-VITRO: LIMITED EFFECT OF DRUGS INTERFERING WITH VIRAL UPTAKE AND INTRACELLULAR TRANSPORT.

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ABSTRACT

BK virus is an important pathogen in kidney transplant recipients. In-vitro studies demonstrated slight anti-viral activity for chloroquine and nystatin. A sialic acid derivative, BTB11968, was identified as a lead compound for further development.
The polyomaviruses are small double stranded DNA viruses, of which the most important species are BK virus (BKV) and JC virus (JCV). BKV reactivation occurs in 10-60 % of kidney transplant recipients, while 1-10% develop viral nephropathy (14). No effective drugs are currently available for the treatment of these patients. This article describes the in-vitro anti-BKV activity of compounds selected for their potential ability to interfere with mechanisms of virus uptake and intra-cellular transport. The anti-viral drug sensitivity assay used is based on direct measurement of BKV (Gardner strain, ATCC # VR837) replication in WI-38 human embryonic fibroblast cells (ATCC#CCL-75) using a 7 day real time PCR assay. Cells are pre-treated with the drug of interest for 2 hours following which virus infection is allowed to occur for an additional 2 hours. Quantitation of a cell gene aspartoacylase provides a measure of cell number and cytotoxicity. Drug cytotoxicity is also evaluated by the conventional neutral red assay. Technical details of these methods have been published (7,13,15). The results are summarized in Table 1 and are further discussed below.

The first therapeutic target to be explored was the viral receptor on host cells. The primary receptor binding determinant on the BKV capsid is the VP-1 protein. The corresponding host ligand is not completely characterized, but appears to contain a terminal α2-3-linked sialic acid associated with Type II gangliosides (3,4,7,8,11). Tunicamycin, a compound known to inhibit synthesis of α(2,3)-linked sialic acids (4), was not effective. We then tested nine sialic acid derivatives selected from the Maybridge HitFinder Collection based on favorable log P values and absence of reactive groups (Figure 1).
BTB11968 or 2-(hydroxymethyl)-6-(nonyloxy)tetrahydro-2H-pyran-3,4,5-triol (Figure 1, compound 1), showed anti-viral activity with 50% effective concentration \( (\text{EC}_{50}) = 6.35 \pm 2.17 \), 50% cytotoxic concentration \( (\text{CC}_{50}) = 126.37 \pm 7.65 \), and neutral red selectivity index \( (\text{SI}) = 30.51 \pm 2.02 \). The PCR assay gave an SI within two fold of the SI determined by the neutral red assay. A decrease in anti-viral efficacy was observed if the standard two hour pre-incubation of cells with the drug was omitted \( (\text{EC}_{50} = 98.93 \pm 18.02, \text{CC}_{50} = 114.5 \pm 27.46, \text{SI} 1.25 \pm 0.51) \), consistent with its presumptive mechanism of interfering with the virus-receptor interaction. All other sialic acid derivatives tested were inactive.

While the sialic acid composition the BKV receptor has now been partly characterized, the associated protein remains undefined. Polyomavirus JC receptor, however, has recently been shown to contain a 5-hydroxy-tryptamine receptor 2A (5HT\(_{2A}\)R) protein component (6). Receptors for 5HT\(_{2A}\) have been described in kidney derived cell lines such as HEK293 cells (9), and in the rat kidney (2), where they modulate cell proliferation. Accordingly, we tested the anti-BKV activity of several known 5HT\(_{2A}\)R inhibitors, namely, chlorpromazine, clozapine, olanzapine and ziprasidone. The results were not encouraging, and indicate that 5HT\(_{2A}\)R associated pathways do not play a major role in the cellular uptake and intra-cellular transport of BKV.

Next, we explored the idea of testing compounds that might interfere with intra-cellular transport of BKV. Viral entry into host cells is mediated by vesicles that have been identified as caveolae (1). The route taken by the virus from cell
membrane to the nucleus includes the endoplasmic reticulum and microtubules 
(5,11). Participation of the Golgi apparatus, and other cytoskeletal elements such 
as actin, and microfilaments has been shown for other polyomaviruses. BKV 
transport into the nucleus is facilitated by nuclear localization signals located on 
BKV proteins VP-2 and VP-3. Since these localization signals are not normally 
exposed on the surface of the virus, partial disassembly of the protein capsid 
must occur in the host cytoplasm prior to viral entry in the nucleus. This 
disassembly occurs, at least in part, within the acidic environment of endosomes 
and lysosomes.

A number of FDA approved compounds with a potential to disrupt intra-
cellular pathways of BKV transport were evaluated. Chloroquine was tested 
because it raises the lysosomal pH, and shows activity against a number of other 
viruses (10,12,16-21). A slight anti-BKV effect was found (SI = 5.32). Notably, 
the EC50 was comparable to that reported by Vincent et al, 2005, for 
coronaviruses. The latter study also showed that chloroquine inhibits 
glysosylation of the SARS coronavirus receptor. However, in our culture system, 
drug treatment of cells prior to BKV infection showed no evidence for blockade of 
the viral receptor (data not shown).

Nystatin, an FDA approved fungal agent was tested for anti-BKV activity 
because of its ability to disrupt caveolae (1). The SI was 5.13 +/- 0.9. 
Amphotericin B, a water soluble functional analog of Nystatin, yielded a 
selectivity index of 2.99. There was a very low margin of safety, as the drug 
concentration just one step higher than the CC50 showed severe cytotoxicity, on
light microscopic examination. Finally, a selectivity index of 3.63 +/- 1.33 was obtained for colchicine, a compound that disrupts microtubules. The EC50 (0.022 µg/ml) is substantially higher than therapeutic concentrations of the drug in patients treated with this compound for gout (0.00075 – 0.0063 µM at a daily dose of 1mg colchicine).

In summary, our testing has identified a compound BTB11968 which appears worth of further evaluation and optimization as an anti-BKV agent (SI=30.5). Of the FDA approved compounds tested, chloroquine has an SI of 5.32, and Nystatin an SI of 5.13. Generally speaking, only drugs with selectivity index >10 are pursued by the pharmaceutical industry for clinical development. However, nystatin and chloroquine are already being used clinically for fungal infections and malaria respectively with an acceptable toxicity profile. Hence, it could be argued, that until better drugs become available, these compounds could be empirically tried in patients with progressive BKV nephropathy. The in-vitro EC50 of chloroquine for several viruses matches the blood concentrations of human subjects under acute anti-malarial treatment (1-15 µM)(10,12,16-21).

Whereas, chloroquine can be administered systemically, Nystatin is suitable only for local use, possibly as bladder wash for hemorrhagic cystitis after bone marrow transplantation.

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REFERENCES


Legend for Figure 1.


All structures were obtained from [http://www.maybridge.com](http://www.maybridge.com)
TABLE 1: Summary of Anti-viral Activity of Tested Compounds*

<table>
<thead>
<tr>
<th>Compound</th>
<th>CC50 (µM)</th>
<th>EC50 (µM)</th>
<th>SI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tunicamycin</td>
<td>0.0473 +/- 0.010</td>
<td>0.039 +/- 0.002</td>
<td>1.23 +/- 0.33</td>
</tr>
<tr>
<td>BTB11967: 2-(hydroxymethyl)-6-(octyloxy)tetrahydro-2H-pyran-3,4,5-triol</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>N/A</td>
</tr>
<tr>
<td>BTB11968: 2-(hydroxymethyl)-6-(nonyloxy)tetrahydro-2H-pyran-3,4,5-triol</td>
<td>126.37 +/- 7.65*</td>
<td>78.3 +/- 11.60</td>
<td>6.35 +/- 2.17</td>
</tr>
<tr>
<td>NRB05157: methyl 3,4,5,6-tetra(acetyloxy)tetrahydro-2H-pyran-2-carboxylate</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>N/A</td>
</tr>
<tr>
<td>NRB05166: 2-(hydroxymethyl)-6-methoxytetrahydro-2H-pyran-3,4,5-triol</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>N/A</td>
</tr>
<tr>
<td>NRB05186: 5-(1,2-dihydroxyethyl-3,4 dihydroxytetrahydrofuran-2-one</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>N/A</td>
</tr>
<tr>
<td>RJC00828: methyl 3,4,5,6-tetrahydroxytetrahydro-2H-pyran-2-carboxylate</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>N/A</td>
</tr>
<tr>
<td>RJC01352: (2R,3R,4R,5S)-3,4,5-triacetoxy-2-(acetyloxymethyl)tetrahydropyran</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>N/A</td>
</tr>
<tr>
<td>RJC01353: (2R,3S,4R,5S)-3,4,5-triacetoxy-2-(acetoxyethyl)tetrahydropyran</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>N/A</td>
</tr>
<tr>
<td>JFD03599: 3,4,5,6-tetrahydroxytetrahydro-2H-pyran-2-carboxylic acid</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>N/A</td>
</tr>
<tr>
<td>Chlorpromazine</td>
<td>10.34 +/- 1.92</td>
<td>7.6 +/- 1.9</td>
<td>1.98 +/- 1.21</td>
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<tr>
<td>Clozapine</td>
<td>40.0 +/- 6.1</td>
<td>23.26 +/- 8.6</td>
<td>4.9 +/- 2.4</td>
</tr>
<tr>
<td>Olanzapine</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>N/A</td>
</tr>
<tr>
<td>Risperidone</td>
<td>&gt;25</td>
<td>&gt;25</td>
<td>N/A</td>
</tr>
<tr>
<td>Ziprasidone</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>N/A</td>
</tr>
<tr>
<td>Nystatin</td>
<td>48.56 +/- 1.49</td>
<td>9.81 +/- 2.01</td>
<td>5.13 +/- 0.9</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>4.99</td>
<td>1.67</td>
<td>2.99</td>
</tr>
<tr>
<td>Chloroquine</td>
<td>8.50 +/- 2.4</td>
<td>1.7 +/- 0.3</td>
<td>5.32 +/- 2.3</td>
</tr>
<tr>
<td>Colchicine</td>
<td>0.0476 +/- 0.017</td>
<td>0.022 +/- 0.009</td>
<td>3.63 +/- 1.33</td>
</tr>
</tbody>
</table>

* EC50 and CC50 refer to the drug concentrations that caused 50% reduction in yield of viral DNA and cellular DNA respectively. SI refers to the ratio CC50/EC50. These determinations were made using a non-linear sigmoidal regression equation as implemented by commercially available software (SigmaPlot 8.0.1, Chicago, IL). All values shown in this table correspond to the PCR assay, except for BTB11968 where the higher neutral red value is also shown. Results with mean +/- sd specified are based on 2-5 experiments. Amphotericin B was tested only once because microscopic examination showed unacceptable cytotoxicity at the next higher dilution tested (6.25µM).