TLR7 Agonist GS-9620 is a Potent Inhibitor of Acute HIV-1 Infection in Human PBMCs

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Introduction

• GS-9620 is an orally bioavailable TLR7 agonist that activates plasmacytoid dendritic cells (pDCs) and B lymphocytes to produce various cytokines including interferon-α (IFN-α)2.

• GS-9620 induced prolonged suppression of hepatitis B virus (HBV) in animal models of chronic infection and is being tested in patients with chronic HBV infection (Phase II).3,4

• GS-9620-induced transient plasma viremia and reduced viral DNA in tissue5,6 was also evaluated in GM-CSF differentiated monocyte-derived dendritic cells that support HIV-1 infection in vitro.

• Herein we investigated the effect of GS-9620 on acute HIV-1 infection in vitro.

Methods

• Anti-HIV-1 activity was assessed in IFN-α-activated human PBMCs and isolated CD4+ T cells in a 5-day multi-cycle HIV-1 infection assay using a HIV-FUSIA readout and at a 3-day single-cycle VSV-G pseudotyped HIV-1 reporter virus (HIV-1VSVg-LUC) infection assay with a luminescence readout. Anti-HIV activity was also evaluated in IFN-α-stimulated peripheral blood mononuclear cells (PBMCs) depleted or mock-depleted (control) from PBMCs by negative selection and flow cytometry (90% depleted in all cases) prior to anti-HIV-1 treatment.

• Conditioned supernatant from GS-9620-treated total PBMCs and pDC-depleted PBMCs were tested for antiviral effect on CD4+ T cells acutely infected with HIV-1.

• GS-9620-induced cytokines were quantified by ELISA (IFN-α) and 29-pia Luminex assays.

• To assess the potency of IFN-α in antagonizing GS-9620-induced anti-HIV-1 activity, blocking antibodies against IFN-α or the IFN-α receptor were added serially during GS-9620 treatment.

• HIV-virus fusion was evaluated in PBMCs and isolated CD4+ T cells using a Bap-AM entry assay7 following pre-treatment with GS-9620 for 2 days.

• Effects of GS-9620 on HIV reverse transcription and integration were evaluated in infected PBMCs by quantitative PCR using primer-probe pairs specific for reverse transcripts (early/late-RT) and integration products.

Figure 1. Assays to Measure Antiviral Activity

Figure 2. GS-9620-induced Anti-HIV Activity is Predominantly pDC-dependent

• GS-9620 is a very (less) biodegradable TLR7 agonist that activates plasmacytoid dendritic cells (pDCs) and B lymphocytes to produce various cytokines including interferon-α (IFN-α).2

Results

Table 1. GS-9620 Exhibits Potent Anti-HIV Activity in PBMC Cultures

<table>
<thead>
<tr>
<th>Target Cell</th>
<th>GS-9620 Treatment</th>
<th>Cell Type</th>
<th>Cytokine</th>
<th>EC50 (nM)</th>
<th>IFN-α (IU/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBMCs</td>
<td>GS-9620</td>
<td>PBMCs</td>
<td>IFN-α</td>
<td>0.02 µM</td>
<td>10000</td>
</tr>
<tr>
<td>PBMCs</td>
<td>DMSO</td>
<td>PBMCs</td>
<td>IFN-α</td>
<td>&gt;2000</td>
<td>27.2 ± 31.0</td>
</tr>
<tr>
<td>CD4+ T cells</td>
<td>GS-9620</td>
<td>CD4+ T cells</td>
<td>IFN-α</td>
<td>0.02 µM</td>
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</tr>
</tbody>
</table>

Conclusions

• GS-9620 inhibits HIV-1 replication in human PBMCs. Antiviral potency was significantly improved upon pre-treatment of culture prior to HIV infection.

• Although GS-9620 was inactive directly in CD4+ T cells, HIV-1 replication was potently inhibited by GS-9620-conditioned PBMC media.

• GS-9620 anti-HIV activity was highly pDC-dependent as depicted by CD1c+ pDCs, but not other PBMC-derived immune cell subsets (NK cells, B cells, CD8+ T cells), significantly reduced GS-9620 antiviral activity.

• IFN-α was detected in GS-9620-treated total PBMC cultures, but not in pDC-depleted cultures. Interferon production was important for GS-9620 antiviral activity as IFN-α/β blocking antibodies greatly diminished GS-9620 antiviral activity.

• Whereas GS-9620 did not affect HIV entry into target cells, GS-9620 and recombinant IFN-α each similarly reduced the levels of early and late HIV reverse transcriptase activities, indicating a replication block at or prior to the RT step.

• Immunomodulatory effects of GS-9620 leading to simultaneous activation of IFN-α expression and inhibition of acute HIV-1 infection are important considerations for its clinical evaluation since the antiviral effect may help restrict potential local spread of virus upon in vivo latency reversal.

References


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