Inhibition of HIV-1 Replication by a Novel Acylguanidine-based Molecule

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Background and Objective

Recent advances in HIV-1 antiretroviral therapy (ART) have substantially reduced morbidity and mortality, but the selection and transmission of drug-resistant strains necessitates ongoing discovery of new antiviral drugs. HIV-1 accessory proteins, including Vpu, enhance viral replication and in vivo pathogenesis and thus may be attractive targets for new classes of antiviral drugs.

Vpu promotes virus release by downregulating the host restriction factor BST-2/Tetherin. Vpu is also reported to be a viroporin (i.e., forming channels in cell membranes), but the role of this activity in HIV-1 replication remains unresolved. A putative inhibitor of Vpu viroporin activity (BIT225) has been described; however, it displays high toxicity in T cell cultures.

We investigated the anti-HIV-1 activity of a novel acylguanidine compound, SM111, which has been shown to inhibit viroporin proteins encoded by Influensa, Dengue, and Hepatitis C viruses.

Materials and Methods

Drugs
- SM111 (lead compound) is a novel acylguanidine-based small molecule
- BIT225 is an acylguanidine-based compound under investigation by Bioston, Ltd (Australia)
- Vemradyne (RAL) is a nucleoside reverse transcriptase inhibitor (NNRTI)
- Eluvirine (EFV) is a non-nucleoside reverse transcriptase inhibitor (NNRTI)
- Indinavir (IDV) is a protease inhibitor (PI)
- Palmitoyl (PAL) is an integrase inhibitor (INI)

Viruses
- Wild type NL4.3 and recombinant NL4.3 strains encoding patient-derived subtype B Pol sequences were used to assess antiretroviral activity of each drug. Different strains harboring major NRTI, NNRTI, PI and INI resistance mutations were chosen in Table I.

Cells
- A GFP reporter CD4+ T cell line (CEM-GFP) and primary PBMCs were used in multi-cycle replication assays to propagate viruses in the presence or absence of SM111 (0-100uM) or other drugs (AZT, EFV, IDV, or RAL; 0-100uM). Infected CEM-GFP cells were quantified by flow cytometry as the % GFP events among viable cells in culture, while p24 Elisa was used to quantify viral growth in PBMCs. CEM-GFP cells were also transfected with WT Vpu or Vpu encoding SM111-selected mutations, and downregulation of CD4 or Tetherin (CD317) monitored by flow cytometry.

Assessment of viral replication

1. CEM cells
- Select G418-resistant T cell line with HIV vector and virology
- Determine % infected cells

2. Human PBMCs
- Culture in the presence or absence of drugs
- Analyze % infected cells

SM111 exhibited limited toxicity in cell lines and PBMCs

Figure 1. Effects of SM111 on cell viability and proliferation. A, CEM-GFP cells were incubated with various concentrations of SM111. Cell counts and viability were determined using ViaCount (Mirepro). No toxicity was observed with up to 100uM SM111 or SM111 (1uM SM111 variant sensitivity), while BIT225 was cytotoxic at >20uM. B, SM111 had minimal effects on cell proliferation in a 6 day assay. C, SM111 is nontoxic in HEK-293 cells up to 100uM. D, SM111 was non toxic in PBMCs up to 50uM, some toxicity was observed at 100uM.

Figure 2. Anti-HIV activity of SM111. A, CEM-GFP cells were infected at MOI of 0.3% and SM111 (various doses) was added at 24hrs. Negative (no drug) wells were used as controls. Viral growth was monitored at days 2 to 9 by flow cytometry. B, HIV-1 replication was inhibited by SM111 in a dose-dependent manner. Results are displayed as % infected cells at day 6. C, HIV-1 replication is not affected by SM111, an acylguanidine analog that has no anti-viroproactivity. Results are displayed as % infected cells at day 6. D, Inhibition of HIV replication in SupT1 cells (cell line lacks BST2/Tetherin) in a multi cycle 8 day assay. E, Inhibition of HIV replication in human primary PBMCs, by 50uM SM111 in a multi cycle 9 day assay.

SM111 inhibits drug resistant HIV-1 strains

Figure 3: SM111 inhibits replication of NRTI, NNRTI, PI and INI resistant HIV-1 strains. A, Replication kinetics of recombinant NL4.3 strains encoding current resistance mutations to current ARVs in the presence of the respective ARV or SM111. B, Spread of drug resistant HIV strains and wild type NL4.3 control in the absence or presence of SM111. Results are displayed as % infected cells at day 6.

SM111 selects mutations in the transmembrane domain of Vpu

Figure 4. Mutations in the Vpu transmembrane (TM) domain are selected in the presence of SM111. A, SM111 resistant HIV-1 variants were generated by passaging NL4.3 in the presence of 100uM drug. B and C, Unique Vpu mutations were observed in three outgrowth strains, resulting in either a 5 AA deletion (strain A), a truncation (strain C) or a substitution (strain H) of the TM sequence.

SM111 inhibits HIV-1 viral egress

Figure 5. Effects of Vpu mutations on Tetherin (CD317)/CD4 downregulation activity, viral spread and SM111 resistance. A, Dot blots showing Tetherin cell surface expression at day 6 post infection. B, % Tetherin and CD4 expression on CEM T cells transfected with wild-type Vpu or Vpu mutants demonstrates impaired downregulation activity by all SM111 selected mutants. C, Vpu mutant viruses displayed enhanced viral spread in the presence of SM111. D, Mutant strains showed a range of susceptibility to SM111, with the SAA deletion (SM111A) remaining most sensitive, truncation (SM111C) moderately sensitive, and 178 substitution (SM111H) least sensitive to the drugs antiviral activity.

HIV-1 Vpu displays partial resistance to SM111 that differs by cell type

Figure 6. NL4-3 Vpu displays partial susceptibility to SM111 that is dependent in part on cell type. A, NL4-3 Vpu displayed minimal resistance to SM111 in CEM-GFP cells but was more resistant to SM111 in CD4+ T cells as shown in (B). C, NL4-3 Vpu replicated poorly in PBMC and displayed minimal resistance to SM111.

SM111 inhibits HIV-1 viral entry

Figure 7. Inhibition of HIV-1 release by SM111. A, Tons of NL4-3 viruses from the supernatants of HEK293 cells harvested at 48hrs post-transfection with pNL4-3 displayed an SM111 dependent-dose decrease. Viral titer was measured using CEM-GFP cells. B, Supernatant p24 concentrations of WT and mutant HIV-1 particles released from HEK293 cells 48hrs post-transfection with virus under different concentrations of SM111.

Conclusions

- SM111 is a novel acylguanidine-based small molecule with broad antiviral activity
- SM111 displays anti-HIV-1 activity with minimal cytotoxicity in T cell lines and PBMCs
- SM111 inhibits the replication of HIV-1 strains resistant to NRTI, NNRTI, PI and INI drugs, indicating a distinct mechanism of action
- SM111 selects for mutations located in the HIV-1 Vpu TM domain that impair Tetherin and CD4 down regulation activity
- Mutant HIV-1 strains and strains lacking Vpu remain at least partially sensitive to SM111, indicating that additional host and/or viral target(s) may be required for drug activity
- SM111 represents a promising lead molecule for future ART studies