differ in glycosylation pattern of viral HA possessed markedly reduced drug susceptibility against NAI in cell culture based assays.

doi:10.1016/j.antiviral.2007.01.079

72

Activation of GS-7340 and Other Tenofovir Phosphonoamidate Prodrugs by Human Proteases

Gabriel Birkus*, Nilima Kutty, Gong-Xin He, Andrew Mulato, William Lee, Martin McDermott, Tomas Cihlar

Gilead Science, 333 Lakeside Drive, Foster City, CA 94404, USA

GS-7340 is an isopropylalaninyl phenyl ester prodrug of a nucleotide HIV reverse transcriptase inhibitor tenofovir (TFV; 9-[(2-phosphonomethoxy)propyl]adenine) exhibiting potent anti-HIV activity and enhanced ability to deliver parent TFV into peripheral blood mononuclear cells (PBMCs) in vivo. The present study focuses on the intracellular metabolism of GS-7340 and its activation by a variety of cellular hydrolytic enzymes. Incubation of human PBMCs in the presence of GS-7340 indicate that the prodrug is more efficiently hydrolyzed to an intermediate TFV-alanine conjugate (Met X) in quiescent PBMCs compared to activated cells. In contrast, the conversion of Met X to TFV and subsequent phosphorylation to TFV-diphosphate occur more rapidly in activated PBMCs. The activity of GS-7340 hydrolase producing Met X in PBMCs is primarily localized to lysosomes and is sensitive to inhibitors of serine hydrolases. Cathepsin A, a lysosomal serine protease has recently been identified as the primary enzyme activating GS-7340 in human PBMCs. Result from the present study indicate that in addition to cathepsin A, a variety of serine and cysteine proteases cleave GS-7340 and other phosphonoamidate prodrugs of TFV. The substrate preferences displayed by the tested proteases towards a series of TFV amidate prodrugs is nearly identical to their relative activities displayed against peptide substrates, indicating that GS-7340 and other amidate derivatives can be considered peptidomimetic prodrugs of TFV.

doi:10.1016/j.antiviral.2007.01.080

73

NIM811, A Cyclophilin Inhibitor, and NM107, An HCV Polymerase Inhibitor, Synergistically Inhibits HCV Replication and Suppresses the Emergence of Resistance In Vitro

Joanna Boerner*, Sue Ma, Teresa Compton, Kai Lin

Novartis Institutes for Biomedical Research, 500 Technology Sq., Cambridge, MA 02139, USA

More effective and better tolerated therapies are needed for chronic hepatitis C which affects 170 million people worldwide. Because of the high heterogeneity and mutation rate of hepatitis C virus (HCV), future therapies are likely to consist of multiple drugs to maximize antiviral efficacy and to prevent resistance. We are taking a two-prong approach to develop novel therapeutic agents for HCV. The first strategy is to target viral proteins such as the NS5B RNA polymerase directly. One such inhibitor, NM283 (valopicitabine), is currently in Phase II clinical trials. The second and a complementary strategy is to target host factors that are also essential for viral replication. NIM811, a cyclophilin inhibitor with potent in vitro antiviral activities, represents such an approach and is under clinical investigation in HCV patients. Here, the combination of NIM811 and NM107 (the active moiety of NM283) was evaluated in vitro using the HCV replicon model as the first step to explore the possibility of using such a combination in patients. HCV replicon cells were treated with various concentrations of the two compounds either alone or in combination. There was a concentration- and time-dependent inhibition of HCV replicon with NIM811 and/or NM107. Importantly, the combination always led to a stronger antiviral effect than either agent alone with no significant increase of cytotoxicity. Moreover, the effect of combination was determined to be synergistic as analyzed in a mathematic model. In addition, drug-resistant clones were generated, and there was no cross-resistance between these two inhibitors of different mechanisms. Furthermore, the frequencies of resistance were determined with the compounds at various concentrations. The barrier to resistance was greatly increased when NIM811 and NM107 were used in combination. In summary, these in vitro results illustrate the significant advantages of combination therapies and warrant exploration of this specific combination in further studies.

doi:10.1016/j.antiviral.2007.01.081

74

Identification of Novel Low Molecular Weight HIV-1 gp41 Fusion Inhibitors Using A New Quantitative High Throughput Fluorescence Intensity Assay

Miriam Gochin [1,2,*], Lifeng Cai [1]

[1] Department Basic Sciences, Touro University—California, Vallejo, CA 94592, USA; [2] Department Pharmaceutical Chemistry, UCSF, San Francisco, CA 94143, USA

A new high throughput screening assay for HIV-1 gp41 inhibitors has yielded novel low molecular weight fusion inhibitors from a peptidomimetic library. Both the assay and the new compounds are described. The assay is performed by mixing two designed peptides with compounds arrayed in multi-well plates, and measuring fluorescence intensity. It can be readily applied to screen large chemical databases for identification of HIV-1 fusion inhibitors. Inhibitors can be detected quantitatively from the assay in three simple steps: (1) a high throughput screen to identify possible positive hits by fluorescence intensity enhancement; (2) a control high throughput screen to eliminate false positives; (3) serial dilution of true positive hits to obtain high throughput dose–response curves for determination of inhibition constants (K_i). The HTS assay has a Z' factor of 0.88 and can rank order inhibitors at 10 μM concentration with K_i's in the range 0.2–30 μM, an ideal range for drug discovery. The assay was validated using known gp41 inhibitors. The applicability