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Increased Pyrophosphorolysis of Mutant HIV RT Enzymes Correlates with Decreased Processivity

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The development of the M184V lamivudine-resistance RT mutation can result in increased zidovudine and adefovir susceptibility for HIV with zidovudine resistance-associated mutations *in vitro* and *in vivo*. An enzymatic analysis of the T215Y and D67N/K70R zidovudine-resistance RT mutations in combination with the M184V RT mutation was undertaken in order to elucidate the mechanistic basis of altered drug susceptibility and altered viral replication kinetics of these common drug-resistant HIV mutants. The K_m, K_i, enzymatic processivity and pyrophosphorolysis activity of *E. coli* expressed site-directed mutant RT enzymes M184V, T215Y, and M184V/T215Y with and without mutations D67N/K70R in the background of HIV strain HXB2D were determined. The purified recombinant mutant RT enzymes had only minor changes compared to the wild-type RT enzyme in the K_m for dNTP substrates and the K_i values for inhibitors adefovir diphosphate and zidovudine triphosphate. These results suggested that differences in the relative binding affinity for these RT inhibitors may play only a small part in the observed drug susceptibility alterations. Changes were observed in analyses of single cycle processivity of the mutant RT enzymes on heteropolymeric RNA templates. The M184V and T215Y single mutant enzymes were less processive than wild-type RT and these mutations were additive in the M184V/T215Y mutant. Similarly, the D67N/K70R/T215Y and D67N/K70R/M184V/T215Y mutant RT enzymes were less processive than wild-type RT. Altered pyrophosphorolysis activity (or reverse nucleotide polymerization) of these mutant RT enzymes was also observed and shown to be inversely related to their processivity such that RT mutants with reduced processivity had increased pyrophosphorolysis. The observed decrease in enzymatic processivity and increased pyrophosphorolysis of the M184V/T215Y–containing RT mutants may contribute to the observed decreased viral replication of M184V/T215Y mutant virus and play a yet unidentified role in the altered drug susceptiblity.

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Viral entry as the primary target of anti-HIV activity of chicoric acid and its tetra-acetyl esters

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The inhibitory effect of L-chicoric acid (L-CA) on the replication of HIV has been attributed to the inhibition of proviral DNA integration (Proc. Natl. Acad. Sci. USA 93: 6326-6331, 1996). Here we show that the primary target of L-CA and its analogues for the anti-HIV activity in cell culture is viral entry. L-CA and D-CA and their tetraacetyl esters were found to inhibit the replication of HIV-1 and HIV-2 strains in MT-4 cells at an EC_{50} of 1.7 to 70.6 µM. In a time of addition experiment L-and D-CA and their tetraacetyl esters were found to interfere with an early event in the viral replicative cycle. Moreover, CA and its analogues did not inhibit the replication of virus strains that were resistant towards polyanionic and polycationic compounds. HIV-1 strains resistant to L- and D-CA were selected in the presence of L- and D-CA. Mutations were found in the V2, V3 and V4 regions of the gp120 of the L-and D-CA-resistant HIV-1 strains. Only a previously described polymorphism mutation was detected in the integrase gene of the resistant strain. Although inhibition of HIV integrase activity by L-CA and its derivatives was confirmed in an oligonucleotide assay, integrase carrying the G140S mutation (which had previously been associated with resistance to L-CA) (J. Virol. 72: 8420-8424, 1998) was inhibited to the same extent as the wild-type integrase. Our results clearly indicate that (i) L-CA owes its antiviral activity to an interaction with the gp120 of HIV, (ii) L-CA interacts with specific amino acid residues in the gp120 molecule, and (iii) the virus overcomes the inhibitory effect of L-CA through mutations in gp120.

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Importance of Stereochemistry in the Design and Selection of Resistance Mutations to D4-Nucleosides. RF Schinazi,[1] B Pai,[2] S Schlueter-Wirtz,[1] SA Martin,[1] PM Tharnish,[1] DC Liotta,[1] CK Chu,[3] JL Hammond,[4] and JW Mellors.[4] Emory Univ/VAMC, Atlanta, GA;[1] Pharmasset Inc, Tucker, GA;[2] College of Pharmacy, Univ of Georgia, Athens, GA;[3] and Univ. of Pittsburgh/VAMC, Pittsburgh, PA.[4]

Nucleosides are versatile molecules with well-known biochemical activation mechanisms. We identified D- and L-D4FC as highly potent inhibitors of HIV-1 and HIV-2. *In vitro* selection with L-D4FC resulted in the rapid appearance of virus exhibiting >20-fold resistance and encoding the M184I/V mutations. In contrast, D-D4FC resistant virus developed only after prolonged selection. Sequencing of the RT from D-D4FC resistant virus identified three previously unreported mutations: K70N, V90I and R172K. A second *in vitro* selection with D-D4FC identified mutations at K65R and V179D. Since these analogs have good virological profiles, but are unstable under acidic conditions, we introduced a vinylic fluoride moiety into the D- and L-nucleosides. The compounds were evaluated against HIV-1 and M184V cloned viruses in human PBM cells. For the L-series, the C, 5-FC, and A derivatives demonstrated significant activity against HIV-1 (EC_{50} < 1.5 µM) with no apparent cytotoxicity in human PBM, Vero, CEM and HepG2 cells. For the D-series, A, I, 6-chloro-2-aminopurine, G, 2,6-diaminopurine, and 5-FC derivatives had selective antiviral activity (EC_{50} < 0.5 µM). Whereas the L-nucleosides were essentially inactive against the 184V variant, most of the D-enantiomers retained their potency. Some of these compounds also had anti-HBV activity in 2.2.15-HepG2 cells without cytotoxicity. The L-2'-fluoro-C analogue inhibited HBV DNA replication at low concentration (EC_{50} = 0.002 µM). The L-2'-fluoro-5-FC analogue was also active. In the D-series the D-2'-fluoro-5-FC analogue (EC_{50} = 0.05 µM) was more potent than the D-2'-fluoro-C analogue (EC_{50} = 2 µM). The potent and selective activity of these chemically stable 2'-fluoronucleosides against the M184V HIV-1 variants and dual antiviral activity against HIV-1 and HBV, suggest that advanced biochemical, pharmacological and animal model studies should be pursued. (Research supported by the NIH and the Department of Veterans Affairs).

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Inhibition of HIV-1 replication by the Cre-loxP hammerhead ribozyme

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An alternative strategy has been the use of antiviral genes that are delivered to uninfected cells as either RNA or DNA and provide intercellular protection from HIV-1. Antiviral genes include those encoding antisense molecules, ribozymes, transdominant proteins, and intracellular antibodies. The potential of such a molecular genetic intervention was examined by using the Cre-loxP recombination system. A recombinant LTR-ribozyme (pBS246, loxP cassette vector) that contains between two loxP sequences was synthesized. The loxP motif was maintained during replication of HIV-1 in COS cells. This type of HIV-1 permissive cells, COS cells was transformed with a Cre expression vector (pBS185) which was shown to encode Cre DNA binding and recombinase activities. HIV-1 (NL-4-3-Luc) co-transfection of COS cells expressing Cre resulted in a substantial reduction in virus replication compared to control cells, and evidence for the presence of the expected excision product was found. Site-specific excision of HIV-1 can therefore be achieved by using the Cre-loxP system with the function of the molecule switch in acute infection.