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Real-time Characterization of the Interaction of HIV-1-Specific Inhibitors with their Binding Site at the HIV-1 Reverse Transcriptase (RT) Using Surface Plasmon Resonance and Biosensor Technology

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Recently several chemically distinct families of compounds have been described that exhibit a unique antiviral specificity for HIV-1. These include the TIBO, HEPT, nevirapine, pyridinone, BHAP, TSAO and α-APA derivatives. Despite their chemical diversity, they all are selective and potent inhibitors of the HIV-1 reverse transcriptase (RT). The stringent structure-activity relationship within each class of compounds points to a highly specific interaction with a putative receptor site ("pocket") on the target enzyme. X-ray crystallographic data, photoaffinity studies, site-directed mutagenesis and analysis of drug-resistant HIV-1 strains have allowed to map this pocket in the immediate vicinity of the catalytic site. Insight in the conformation of this pocket during enzyme inhibition may help designing second generation RT inhibitors and/or inhibitors with an expanded antiviral spectrum. Most of the current knowledge stems from either static data (X-ray crystallography) or data obtained after system equilibration (equilibrium dialysis, enzyme Ki/Km). We have now attempted to analyze the interaction of the inhibitors with HIV-1 RT in real time. This biospecific interaction analysis (BIA) was based on the surface plasmon response (SPR) principle used in the BIAcore™ system (Pharmacia). A new α-APA derivative containing a primary amino group was synthesized. This compound could be covalently bound to the hydrogel surface of a sensor chip functioning as a biospecific interface. After optimization of the immobilization procedure we were able to carry out real-time measurements of the mass changes at the sensor surface in the presence of RT. Interactions with drug-resistant RT and competing ligands could be studied. As it permits the analysis of biospecific real-time interactions with mutant enzymes and/or competing inhibitors, this new technology should contribute to our understanding of the exact interaction between the different amino acids in and around the drug-binding pocket and the different classes of RT inhibitors.

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Characterization of Human Immunodeficiency Viruses Resistant to Oxathiolane-Cytosine Nucleosides. R. F. Schinazi,[1]* R. M. Lloyd, jr.,[1] M.-H. Nguyen,[2] D. Cannon,[1] N. Ilksoy,[1] C. K. Chu,[3] D. C. Liotta,[1] and J. W. Mellors.[2] VA Medical Center/Emory University, Decatur, GA 30033;[1] VA Medical Center/Department of Medicine, University of Pittsburgh, Pittsburgh, PA 15261;[2] and Department of Med. Chem. and Pharmacognosy, College of Pharmacy, Univ. of Georgia, Athens, GA 30602, USA[3]

The (-)-enantiomers of 2',3'-dideoxy-5-fluoro-3'-thiacytidine [(-)-FTC] and 2',3'-dideoxy-3'-thiacytidine [(-)-BCH-189] were recently shown to inhibit selectively HIV-1, HIV-2, and hepatitis B virus *in vitro*. The potential for HIV resistance to these compounds was evaluated by serial passage of the virus in primary human lymphocytes and MT-2 cells in the presence of increasing drug concentrations. Highly drug-resistant viral variants dominated the replicating virus population after 2 or more cycles of infection. The resistant variants were cross-resistant to (-)-FTC, (-)-BCH-189, and their (+)-congeners, but remained susceptible to DDC, AZT, 3'-fluoro-3'-deoxythymidine, DDI, PFA, and the TIBO compound R82150. HIV-1 reverse transcriptase (RT) derived from drug-resistant viral particles was 25- to 50-fold less susceptible to the 5'-triphosphates of FTC and BCH-189 compared with enzyme from parental drug-susceptible virus. DNA sequence analysis of the RT gene amplified from resistant viruses consistently identified mutations at codon 184 from Met (ATG) to Val (GTG or GTA) or Ile (ATA). Sequencing analysis of amplified RT from a patient who had received (-)-BCH-189 therapy for 5 months demonstrated the Met^{184} to Val (GTG) mutation, indicating that this change can occur *in vivo*. The Met^{184} residue lies in a highly conserved polymerase motif (Tyr-Met-Asp-Asp) adjacent to the putative catalytic site of the HIV-1 RT comprised of the carboxylate triad Asp^{110}, Asp^{185}, and Asp^{186}. Substitution of the Met^{184} residue appears to markedly affect the anti-HIV activity of oxathiolane-cytosine analogs.These findings should permit effective monitoring for the emergence of resistance to these drugs.