77

Antiviral Resistance to Enantiomers of 2',3'-Dideoxycytidine Analogues — Common Threads. R. F. Schinazi,[1]* R. M. Lloyd, Jr.,[1] A. McMillan,[1] G. Gosselin,[2] J.-L. Imbach,[2] C.K. Chu,[3] and J.-P. Sommadossi.[4] VA Medical Center/Emory University, Decatur, GA 30033;[1] University of Montpellier II, 34095 Montpellier, France,[2] Dept. of Medicinal Chemistry, College of Pharmacy, University Georgia, Athens, GA 30602,[3] and University of Alabama, Birmingham, AL 35294,[4] USA.

The development of HIV-1 resistant viruses to (-)-β-L-2',3'-dideoxy-3'-thiacytidine [3TC] in cell culture and in humans was first described by our group. These variants were highly (> 1,000-fold) resistant to 3TC, but remained susceptible to AZT and HIV-1 specific inhibitors. There has been no previous report of development of resistant viruses to (-)-β-D-2',3'-dideoxy-5-fluoro-3'-thiacytidine [D-FTC] and (-)-β-L-2',3'-dideoxycytidine [L-FddC], compounds related structurally to 3TC. D-FTC and L-FddC are cross-resistant with 3TC and (-)-β-L-2',3'-dideoxy-5-fluoro-3'-thiacytidine [L-FTC], although the level of cross-resistance with D-FTC was lower (< 500-fold). Highly resistant viruses were selected in primary human peripheral blood mononuclear cells infected with HIV-1_{LAI}. DNA sequence analysis of the RT gene amplified from resistant viruses consistently identified mutations at codon 184 from Met (ATG) to Val (GTG) for both D-FTC and L-FddC. From these data, we infer that the L-configuration is important for conferring high level resistance to oxathiolane and 2',3'-dideoxycytosine nucleosides at codon 184. However, lower level resistant virus with the same genetic composition can be obtained with certain D-nucleosides. We have also successfully prepared and characterized phenotypically and genotypically for the first time L-FTC-resistant simian immunodeficiency virus (SIV); the M184V mutation was also obtained with this virus. Studies on the pathogenesis of this SIV variant in non-human primates are planned. *(Support: DOD, VA, CNRS/ISERM, and NIH).*

78

Kinetic Analysis of Human Immunodeficiency Virus Type-1 Reverse Transcriptase with Mutations at Methionine 184 in the Highly Conserved YMDD Region. JE Wilson[1], B Caligan[1], A Aulabaugh[2], S McPherson[3], JK Wakefield[3], S Jablonski[3], CD Morrow[3], PA Furman[2], and JE Reardon[1]. [1]Division of Biochemistry and [2]Division of Virology, Burroughs Wellcome Co., Research Triangle Park, NC, USA, and [3]Department of Microbiology, University of Alabama at Birmingham, Birmingham, Al, USA.

Mutations at methionine 184 in the highly conserved YMDD region of HIV-1 reverse transcriptase have an effect on both viral replication rates and inhibition of viral replication by 1-β-L cytidine analogs. We have previously reported that 1) recombinant M184A RT showed increases in the K_m values for dTTP and dCTP during processive RNA-directed synthesis, and 2) the mutation M184V caused significant increases in K_i values to 1-β-L cytidine analogs. In this study, we present additional evidence that this region is important in catalysis. Steady state enzyme assays were done with the purified wt and variant HIV-1 RT M184A and M184V. M184A RT had a 40-fold increase in the K_m value for dCTP using single turnover conditions during RNA-directed DNA synthesis but not during DNA-directed DNA synthesis. No change in k_{cat} was observed. This indicated that there was a change in the K_d or the k_p induced by the mutation. We also examined the effect the mutation M184V had on the K_i values of 1-β-D and 1-β-L adenosine, guanidine, and thymidine nucleoside analogs. Significant increases in K_i values were observed. Presteady state kinetics was used to analyze the mechanism for 1) the increase in K_m value seen with M184A RT during single-turnover RNA-directed synthesis, and 2) the increase in the K_i values for 1-β-L nucleoside analogs seen with M184V RT.