83

Kinetic Analysis of the Interaction of Inhibitors with RMuLV and Human Immunodeficiency Virus (HIV) Reverse Transcriptases (RTs). J.M. Cherrington[1], S.J.W. Allen[1], S.C. Kunder[2], M.A. Ussery[2] and M.S. Chen[1]. [1]Gilead Sciences, Inc., Foster City, CA. 94404; [2]FDA, Rockville, MD 20857.

RMuLV has been pursued as a murine antiretroviral drug screening model for AIDS. AZT and PMEA have been shown to provide antiviral protection in mice infected with RMuLV. The objective of this study was to determine the inhibitory effects of several antiretroviral compounds on RMuLV and HIV RTs. The Km and Ki values for HIV and RMuLV RTs are similar when using DNA as template. However, HIV RT has much lower Km and Ki values when using RNA as a template in comparison with RMuLV RT.

	Km (μM)			Ki (μM)				
HIV RT	dATP	TTP	dCTP	PMEApp	AZTTP	ddCTP	HPMPCpp	FIAUTP
DNA[a]	4.6	2.1	4.6	0.98	0.51	0.53	20.8	3.62
RNA-1[b]	0.05	0.09	0.14	0.012	0.008	0.054	0.826	0.078
RNA-2[c]	n d	2.6	n d	n d	0.012	n d	nd	0.066

	Km (μM)			Ki (μM)				
RMuLV RT	dATP	TTP	dCTP	PMEApp	AZTTP	ddCTP	HPMPCpp	FIAUTP
DNA[a]	3.5	3.2	2.5	0.81	0.38	3.02	13.5	1.40
RNA-1[b]	2.2	3.7	2.1	0.39	0.89	8.6	11	0.98
RNA-2[c]	n d	37.7	nd	n d	0.183	n d	n d	0.91

[a] activated calf thymus DNA primer template. [b] defined sequence synthetic RNA template (81 nucleotides) annealed to DNA primer (15 nucleotides). [c] poly $r(A).d(T)_{12-18}$ primer template. nd, not determined.

84

Cloning, Purification and Biochemical Characterization of HIV-2 Reverse Transcriptase (RT) and Comparison with Endogenous HIV-2 RT.

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Two-thirds of all estimated AIDS cases have occurred in Africa where, especially in the western area, the HIV-2 infection is rapidly spreading. A number of cases have also been reported in Europe and India.

The HIV-2 RT is a crucial enzyme for virus inhibition, being the target of the nucleoside analogues currently used for AIDS therapy. As in the case of HIV-1, the HIV-2 RT can be expected to be involved in drug resistance. These considerations led us to set up an easy method to clone HIV-2 RT from provirus.

Two primers have been designed to amplify by PCR the segment of the pol gene (from nucleotide 2367 to nucleotide 4071 of the ROD strain) encoding for an HIV-2 RT with one additional amino acid at the N-terminus. The PCR fragment was digested and cloned into the expression vector p*Trc*99A; the enzyme was expressed in E.Coli JM109 and purified via ion exchange chromatography.

Optimal assay conditions relating to pH, KCl, $MgCl_2$, $MnCl_2$ concentrations have been defined for the HIV-2 rRT. The biochemical characterization of the enzyme, its interaction with several inhibitors, together with parallel studies on virionic RT, will be reported.

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