33

SELECTIVE INHIBITION OF VIRAL DNA POLYMERASES BY THE TRIPHOSPHATES OF ß-L-2',3'-DIDEOXYCYTIDINE (ß-L-DDC) AND ITS 5-FLUORO DERIVATIVE (ß-L-FDDC). A. Faraj[1], R.F. Schinazi[2] G. Gosselin[3],J-L Imbach,[3] and J.P. Sommadossi[1]. [1]University of Alabama at Birmingham, Birmingham, AL 35294, USA; [2]VA Medical Center/ Emory University, Decatur, GA 30033, USA; and [3]University of Montpellier II, 34095 Montpellier, France.

ß-L-2',3'-Dideoxycytidine (ß-L-ddC) and its 5-fluoro derivative (ß-L-FddC) have potent and selective antiviral activity against human immunodeficiency viruses type 1 and type 2 and hepatitis B virus *in vitro*. The 5'-triphosphates of ß-L-ddC and ß-L-FddC were synthesized and inhibition of HIV-1 reverse transcriptase (RT) and human DNA polymerases α, ß and γ by these derivatives was studied. Using a poly $(rI)_n$.oligo$(dC)_{10-15}$ as a template primer, ß-L-ddCTP and ß-L-FddCTP competitively inhibited HIV-1 RT, with an inhibition constant of 1.3 µM and 1.0 µM, respectively, with respect to dCTP. ß-L-ddCTP and ß-L-FddCTP did not inhibit human DNA polymerases α, ß, and γ up to 50 µM. In contrast, ß-D-ddCTP inhibited HIV-1 RT with an inhibition constant of 0.4 µM, but potently inhibited DNA polymerase γ and also competitively inhibited DNA polymerase ß with a K_i of 0.5 µM, with respect to dCTP. The effects of ß-L-ddCTP and ß-L-FddCTP on HBV DNA polymerase will be reported. The potent activity of ß-L-ddCTP and ß-L-FddCTP against viral DNA polymerases and their lack of inhibition against human host DNA polymerases, suggests that further development of these compounds for treatment of HIV-1 and HBV infections merits consideration.

34

Didanosine (DDI) and Zidovudine (AZT) Sensitivities of HIV-1 Strains Isolated from DDI-Treated Patients, and Tested in a HeLa-CD4 Immunofluorescence Focus Assay (IFFA). Ch.Murrenhoff, J.P.Kruppenbacher, H.J.Eggers, Institut fuer Virologie, Universitaet zu Koeln, D-50935 Koeln, Germany

First passage HIV isolates (detected by p24 Ag) were grown in peripheral blood leukocytes without polybrene. Cell-free stock virus was harvested from the supernate. Medium of HIV infected HeLa-CD4 monolayers was supplemented with different drug concentrations and with DEAE dextran. Three days p.i. single cells were fixed on glass slides, stained by indirect immunofluorescence using anti-HIV-positive human serum, and fluorescent foci were counted. The IFFA produced no false positive results in contrast to HeLa-CD4 plaque assays: "plaques" (stained with crystal violet) were regularly seen also in uninfected controls. The variation coefficients of drug sensitivity values were ≤1.0. DDI sensitivity of HIV isolates from 5 patients (2 ARC, 3 AIDS; 2-12 months before receiving AZT for 9-16 months) ranged from 2.2-4.4µM ID_{50} before therapy. DDI sensitivity decreased in two patients (2.2→12.3, 2.5→9.1µM ID_{50}) who had received high doses of DDI. No significant change of DDI sensitivity was observed in 3 patients receiving low doses of DDI. A relation between DDI-ID_{50} and clinical parameters was not apparent. The highest AZT-ID_{50} (0.181µM) was detected in a patient who had discontinued AZT therapy 2 months before. After treatment with DDI for 20 weeks the AZT-ID_{50} decreased to 0.029µM (the DDI sensitivtiy remained unaffected). 7/12 (58%) CPE-positive isolates (80% of all isolates are CPE-positive) could be tested by HeLa-CD4 IFFA in contrast to CPE-negative isolates. HeLa-CD4 IFFA appears to be a reliable method to monitor drug sensitivity, though applicable to only a limited number of clinical HIV isolates. Testing of further 190 isolates from 39 DDI-treated patients is under way.