99

Study of Human Immunodeficiency Virus Resistance to the Acyclic Nucleoside Phosphonate Analogs. A. Fridland, P. Aduma, N. Sarkissian, R.V. Srinivas. Department of Infectious Diseases, St. Jude Children's Research Hospital, Memphis, TN 38101.

The acyclic 9-(2-phosphonylmethoxyethyl) adenine (PMEA) is one of a new class of broad spectrum antiviral agents. PMEA selectively inhibits HIV-1 replication and is currently being evaluated as a potential antiAIDS drug in patients. We have investigated the efficacy of PMEA and a related derivative 9-(2-phosphonylmethoxypropyl) diaminopurine (PMPDAP) against HIV isolates exhibiting various degrees of resistance to zidovudine. Clinical HIV isolates highly resistant (~ 50-200-fold) to ZDV were found to be ~ 2-8-fold less susceptible to PMEA and PMPDAP. In contrast, a panel of HIV isolates showing intermediate levels (~ 8 to 25-fold) of ZDV resistance did not exhibit any detectable cross-resistance to PMEA (1.4 uM vs 0.8 to 1.0 uM). HIV-1 was also serially passed in MT-2 cells in increasing concentrations of PMEA, PMPDAP, and 9-(2,5-dihydro-5-phosphonomethoxy)-2-furanyl adenine (D4AMPI) to define the emergence of viral resistance to the phosphonate analogs. No emergence of drug resistance was demonstrated for either PMEA or PMPDAP after about 12 serial passages. However, in the case of D4AMPI a decrease in sensitivity of HIV of about 6-fold occurred after 12 passages in the presence of the drug. These differences in resistance profile of the phosphonate analogs may help in elucidating the molecular mechanism of action of these compounds and support the speculation that resistance to the phosphonate analogs may not emerge as quickly as with other anti-HIV drugs.

100

Enzymatic phosphorylation of 9-(2-phosphonylmethoxyethyl) adenine (PMEA) in Drug sensitive and Resistant Cells. BL Robbins, MC Connelly, JJ Greenhaw, A. Fridland. Department of Infectious Diseases, St. Jude Children's Research Hospital, Memphis, TN 38101.

PMEA is an adenine phosphonate analog which is undergoing Phase I/II clinical evaluation for HIV treatment in AIDS patients. In order to be active, PMEA must first be activated to the diphosphate derivative but the exact pathway involved is still unclear. We induced resistance to PMEA in the human lymphoid T cell CEMss by exposing the cells to increasing concentrations of the drug. The IC50 value increased ~ 200-fold in the resistant variant (CEMr-1). CEMr-1 is cross-resistant to various phosphonate analogs such as phosphonylmethoxyethyl diaminopurine and the lipophilic prodrug bispom PMEA, and partially cross resistant to nucleoside analogs such as 2-fluoro arabinosyladenine. The phosphorylation of PMEA was inhibited by greater than 50% in the CEMr-1 cells. Using ATP as the phosphate donor extracts of CEM cells phosphorylated PMEA to its mono- and diphosphorylated derivative while the mutant was significantly deficient in this phosphorylation. No other nucleotide displayed appreciable activity as phosphate donor. Subcellular studies demonstrated two different phosphorylating activities for PMEA in CEM cells, with the major activity (~ 70%) localized in the mitochondria and the remainder in cytosol. In the resistant CEMr-1 cells, the deficiency in PMEA phosphorylation was associated with the mitochondria enzyme activity. Treatment of CEM extract with DTNB and PHMB, two inhibitors of type 1 adenylate kinase (AK1) and pyrimidine nucleoside monophosphate kinase, did not affect the phosphorylation of either AMP or PMEA, but did inhibit that of UMP and HPMPC. These results show that there are different pathways for activation of the different phosphonate analogs but the major route for activation of PMEA and its analogs is via AK2, an enzyme which is localized in the intermembrane space of mitochondria.