94

Activation of 2'-fluoro-2',3'-dideoxyarabinosyladenine (FddA), an Anti-HIV Agent, in Both Quiescent and PHA-Activated Human Peripheral Blood Mononuclear Cells (PBMC). H-T. Ho, K.L. Woods, M. Mansuri and M.J.M. Hitchcock, Bristol-Myers Squibb Pharmaceutical Research Institute, Wallingford, CT. USA.

Human immunodeficiency virus type-1 (HIV-1) infection of quiescent T-lymphocytes leads to production of unintegrated, proviral DNA which becomes integrated into the host genome after mitotic stimulation. However, anti-HIV drugs dependent on thymidine kinase activation (e.g. Azidothymidine) are not phosphorylated in quiescent PBMC. Phosphorylation of the anti-HIV compounds, FddA and FddI, in CEM cells (lymphocytic), U-937 cells (monocytic) and PBMC which were quiescent or stimulated with PHA was measured by SAX anion exchange HPLC of methanol extracts. FddATP was formed from FddA in quiescent and stimulated PBMC, and in CEM and U-937 cells. Formation of FddATP from FddI was also shown for quiescent PBMC and CEM and U-937 cells. After a 6 h exposure to FddA and its removal from the medium, the half life of FddATP was about 20 h in both quiescent PBMC and U-937 cells. This slow intracellular decay of FddATP after removal of free FddA suggests that the inhibitory effect would be long lasting in vivo. Also, the formation of FddATP in quiescent PBMC suggests FddA might inhibit provirus formation in these cells in vivo, and provides a rationale for the use of anti-HIV nucleosides which do not use cell-cycle dependent enzymes for their phosphorylation.

95

Structure and Activity Correlation-Studies of Anti-HIV Acyclic Guanine Nucleotide Analogs at Enzyme Level. H-T. Ho, H. De Boeck, K.L. Woods, S.A. Konrad, V. Brankovan, M.J.M. Hitchcock and R. Datema., Bristol Myers-Squibb Pharmaceutical Research Institute, Wallingford, CT. USA.

Three Anti-HIV acyclic guanine nucleotide analogs and their diphosphates were studied enzymatically to understand the mechanisms of action of these compounds at the molecular level: 9-(2-phosphonylmethoxyethyl)guanine (PMEG, 1), (R)- and (S)-9-((2-phosphonylmethoxy)-1-propyl)guanine ((R)- and (S)-2'MePMEG, 2 and 3). In vitro screening against HIV activity gave IC_{50} values of 0.7, 1.5 and 17 uM for compound 1, 2 and 3 respectively. Cell-doubling time increased by 100% with 1.5 uM of 1, 340 uM of 2 and 120 uM of 3, indicating that 2 was the most selective agent. Phosphorylation of these compounds by GMP kinase to their monophosphates took place at 0.1 (1), 0.2 (3) and 1.6% (2) of the efficiency ($(Vm/Km)_{drug}/(Vm/Km)_{GMP}x100\%$) of that of GMP. $Ki_{inhibitor}/Km_{dGTP}$ ratio obtained from assaying HIV-reverse transcriptase were 0.269, 0.144 and 1.074 for PMEGpp, (R)-2'MePMEGpp, and (S)-2'MePMEGpp respectively. Thus the (R)-2'MePMEGpp is a more potent inhibitor of the HIV-RT than the (S)-form, which is in accordance with the (R)-2'MePMEG being the more potent anti-HIV agent. Studies using host-cell DNA polymerase are underway to determine the basis of selectivity of (R)- and (S)-2'MePMEG relative to PMEG.