75

Dipyridamole inhibits HIV-1 and potentiates the antiviral activity of dideoxynucleoside drugs in cultured cells: biochemical mechanisms of action. J. Szebeni*, S. Patel*, O.S. Weislow**, G. Betageri*, S.M. Wahl***, L.M. Wahl***, J.N. Weinstein** National Cancer Institute (NCI), ***National Institute of Dental Research, NIH, Bethesda, MD, **NCI-Frederick Cancer Research Facility, Frederick, MD, USA

Dipyridamole (DP) is a widely used coronary vasodilator and antiplatelet drug. We find that it has intrinsic anti-HIV activity and markedly potentiates (5- to 10-fold) the antiviral effects of azidothymidine (AZT) in cultured human monocyte/macrophages (M/M), stimulated T-lymphocytes and CEM T-lymphoblastoid cells. In M/M DP also potentiates the antiviral activity of dideoxycytidine, and in CEM cells it antagonizes the cytotoxic effect of AZT (PNAS 86:3842, 1989). Correlative studies with uninfected cells revealed complex effects of DP on the transport of ^{3}H-dideoxynucleosides (ddNs) and on the intracellular appearance of the phosphorylated derivatives of these drugs and the corresponding physiological deoxynucleosides (dNs), thymidine (dThd) and cytidine. Most importantly, DP differentially inhibited uptake of dNs and intracellular appearance of their phosphorylated derivatives. Hence, it may suppress the antagonistic influence of dNs on the antiviral efficacy of ddNs. The data suggest a direct effect of DP on the phosphorylation and/or dephosphorylation process. In CEM cells, in the presence of dThd, DP suppressed the phosphorylation of AZT. This action could underlie the protective action of DP against the toxicity of AZT in these cells.

76

Cellular Pharmacology of 5',2-Anhydro-3'-azido-3'-deoxythymidine. A Potent and Selective Inhibitor of HIV-1 Replication. E.M. August and W.H. Prusoff, Dept. of Pharmacology, Yale University School of Medicine, New Haven, CT, USA.

The 5',2-anhydro analog of 3'-azido-3'-deoxythymidine (AZT) has been shown to have significant anti-HIV-1 activity with an IC_{50} value of 0.56uM in CEM-F cells infected with the HIV/LAV strain of HIV-1. Moreover, anhydro-AZT (anAZT) was significantly less toxic to CEM-F cells than AZT. In order to ascertain whether anAZT acts as prodrug of AZT, or whether the compound may possess antiviral activity per se, we have synthesized [methyl-^{14}C]anAZT and studied its metabolism in H9 cells, a human lymphocytic cell line. After exposure of H9 cells to 10uM [^{14}C]anAZT for 1 hr, approximately 50% of the intracellular radioactivity was present as AZTMP, with a low level of formation of AZTTP. These levels remained relatively stable out to 24h of incubation. Depending upon the procedure for preparation of cell-free extracts of H9 cells, subsequent incubation with 10uM [^{14}C]anAZT produced either small amounts or no AZTMP, even though such systems are capable of metabolizing AZT. anAZT is stable over a range of pH's encountered in cells, with half-lives of > 50h in the pH range 4.5-7.4. Furthermore, no AZT formation was detected in the extracellular media after incubation of anAZT with H9 cells, indicating that the anhydro compound is taken up intact by the cells. These data suggest either intracellular hydrolysis of anAZT to AZT followed by rapid phosphorylation, or a phosphorolytic cleavage. The mechanism involved is under investigation. Thus, the metabolism of the 5',2-anhydro analogs may involve a unique mechanism of activation.
(Supported by NCI grants CA-05262 and CA-45410, and NIAID grant AI-26055).