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Inhibition of de novo pyrimidine pathway by 3'-azido-3'-deoxythymidine in human bone marrow cells: rationale for uridine "rescue". J.P. Sommadossi, A. Faraj and F. Naguib. University of Alabama at Birmingham, Birmingham, AL 35294.

We have previously demonstrated that uridine reverses the toxicity of 3'-azido-3'-deoxythymidine (AZT) in human granulocyte-macrophage colony forming units (CFU-GM) *in vitro* without impairment of antiretroviral activity (AAC 32:997-1001, 1988). We recently developed novel liquid suspension cultures of human pluripotent progenitor cells (CD34+). After 14 days most cells had differentiated to the granulocyte-macrophage lineage and toxicity experiments with AZT showed a good correlation with clonogenic assays (AAC 31:452-454,1987). Using these cultures 100 μM uridine completely protected from toxicity induced by 5 μM AZT the IC_{50}. Cells exposed for 14 days to toxic concentrations of 1-10μM of AZT were incubated for 24 hrs with 20 μCi [^{14}C]- orotic acid (OA) methyl ester which permited detection of most nucleotide pools including OMP, UMP, UDP, UTP, CTP, and TTP. Accumulation of OA and orotidine combined with a decrease (80%) of UMP where the most dramatic alterations in 10μM AZT-treated cells. Subsequent nucleotides derived from UMP were also affected to varying degrees. In contrast, AZT monophosphate (1mM) had no effect on cytosolic mammalian orotate phosphoribosyl transferase (OPRT) or orotidylate decarboxylase (ODC).These data suggest that AZT down-regulates OPRT and ODC activity and/or leads to unfunctional enzymes. Therefore, uridine rescues AZT toxic effects through the salvage pathway preferentially used by the cellular machinery. (PHS Grant HL-42125)

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Phosphorylation of HIV-1 Reverse Transcriptase Modulates its Enzymatic Activity. N. Cheng, C. A. Ohmstede, M. St. Clair, P. A. Furman. Wellcome Research Laboratories, Research Triangle Park, NC, 27709, USA.

Phosphorylation of proteins has been shown to modulate the activities of many important enzymes, among these are eukaryotic DNA and RNA polymerases. We have investigated the phosphorylation of HIV reverse transcriptase (RT) in vitro and in virally infected CEM cells. In vitro, HIV RT was shown to be a substrate for cAMP-dependent protein kinase (PKA). The stoichiometry of the reaction was shown to be one phosphate to one RT monomer, therefore both subunits were phosphorylated to the same extent. RT, purified from an E. coli expression system and phosphorylated by PKA, was about 9-fold more active than unphosphorylated or dephosphorylated RT in the presence of 100 mM KCl. Reverse transcriptase in CEM cells infected with HIV was found to be phosphorylated when cells were labeled with ^{32}P. The site of phosphorylation was on a Ser residue, as was observed with PKA phosphorylated RT in vitro. When RT isolated from intact virions was treated with phosphatase, reverse transcriptase activity decreased about 10-fold, consistent with the phosphorylation-dephosphorylation relationship observed in the in vitro PKA phosphorylation model. Thus we believe that cell-mediated phosphorylation of RT may play an important role by modulating the in vivo reverse transcriptase activity.