73

AZT Metabolism and Toxicity: Application of 5-Azido-3'-Azido-2',3'-Dideoxyuridine Monophosphate, a Photoaffinity Analog of AZT-Monophosphate. R. R. Drake*, L. J. Underwood*, R. Jones*, E. Battaglia^, F. Mao*, T. M. Rechtin*, A. Cantu#, S. Anderson#, A. Radominska^, and M. P. Moyer#.Depts of Biochemistry* and Medicine^, U.A.M.S., Little Rock, AR 72205; Dept. of Surgery#, U.T.H.S.C., San Antonio, TX 78284.

Base-substituted azido-nucleotide photoaffinity analogs have routinely proven to be effective tools for identifying, charcterizing and mapping active-site peptides of nucleotide utilizing enzymes. Therefore, we have synthesized and tested 5-azido-3'-azido-2',3'-dideoxyuridine, which is a potential photoaffinity analog of two HIV drugs, AZT and 3'-azido-2',3'-dideoxyuridine (AZddU). Bacterially expressed HSV-1 thymidine kinase and [γ-^{32}P]ATP were used to make [32P]5-azido-3'-azido-2',3'-dideoxyuridine monophosphate ([^{32}P]5N_3AZddUMP). The photoaffinity properties of this analog were then tested with the HSV-1 thymidine kinase. Photoaffinity labeling of this enzyme was saturable (half maximal 30 μM) and could be specifically inhibited by AZT, AZTMP, thymidine and TMP. Since AZTMP has been reported to inhibit nucleotide sugar transport and subsequent glycosylation reactions, rat liver microsomal membranes were photolabeled with [^{32}P]5N_3AZddUMP. Based on inhibition experiments with AZTMP and UDP-sugars, potential nucleotide translocating proteins have been identified in these membranes. Additionally, 0.1 mM AZTMP inhibited translocation of UDP-glucuronic acid by 40% in these membranes. To begin to examine AZT toxicity of mitochondria, human liver mitochondria were prepared and photolabeled with [^{32}P]5N_3AZddUMP. Two types of photolableled proteins were identified: two proteins of 120 kDa and 65 kDa were specifically inhibited with AZTMP, but not TMP; two proteins of 80 kDa and 47 kDa were inhibited by AZTMP, TMP and dCMP. Initial results suggest specific interactions of [^{32}P]5N_3AZddUMP (and therefore AZTMP) with mitochondrial proteins that may be involved in pyrimidine nucleotide translocation. Lastly, the anti-HIVactivity of 5N_3AZddU was determined using HIV- IIIB in H9 cells. At 1 μM, 5N_3AZddU was 10-fold less effective than AZddU and 50-fold less effective than AZT. Cumulatively, 5N_3AZddU and 5N_3AZddUMP have been shown to act as effective biological mimics of AZT and AZTMP and thus should prove useful in studying enzymes involved in AZT metabolism.

74

***In Vitro* Potency of Inhibition by Antiviral Drugs of Hematopoietic Progenitor Colony Formation Correlates with Exposure at Hemotoxic Levels in HIV Positive Humans.** R. E. Dornsife and D. R. Averett, Burroughs Wellcome Co., Research Triangle Park, North Carolina, U.S.A.

The ability of eight antiviral nucleoside drugs (alovudine, zidovudine, zalcitabine, didanosine, stavudine, lamivudine, ganciclovir, and acyclovir) to inhibit *in vitro* colony formation of human hematopoietic progenitors (CFU-GM, BFU-E) was measured. The estimated inhibitory potency (I_{50}) was calculated from an average of several independent experiments with marrow from healthy donors. The I_{50} values for this set of drugs varied widely from one drug to another in this standardized assay system, ranging from 0.1 μM to > 400 μM. The human exposure to each drug (expressed as area under the plasma concentration-time curve, $AUC_{0\text{-}24}$) which inhibited hematopoiesis was estimated from clinical toxicologic and pharmacokinetic data. The $AUC_{0\text{-}24}$ at the hemotoxic dose in HIV patients correlated well with the *in vitro* potency of inhibition (I_{50}). This correlation allows estimation of the dose at which bone marrow toxicity may occur in patients chronically treated with candidate antiviral drugs.