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Comparative Metabolism of the Antiviral Dimer AZT-P-ddI and the Monomers Zidovudine and Didanosine by Rat, Monkey and Human Hepatocytes

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AZT-P-ddI is an antiviral heterodimer composed of one molecule of 3'-azido-3'-deoxythymidine (AZT) and one molecule of 2',3'-dideoxyinosine (ddI) linked through their 5' positions by a phosphate bond. The metabolic fate of the dimer was studied using isolated rat, monkey and human hepatocytes and was compared with that of its component monomers AZT and ddI. Upon incubation of double labeled [^{14}C]AZT-P-[^{3}H]ddI in freshly isolated rat hepatocytes in suspension at a final concentration of 10 μM, the dimer was uptaken intact in cells and then rapidly cleaved to AZT, AZT monophosphate, ddI and ddI monophosphate. AZT and ddI so formed were then subject to their respective catabolism. Extra- and intracellular HPLC analyses revealed the presence of unchanged dimer, AZT, 3'-azido-3'-deoxy-5'-β-D-glucopyranosylthymidine (GAZT), 3'-amino-3'-deoxythymidine (AMT), ddI and a previously unrecognized derivative of the dideoxyribose moiety of ddI, designated ddI-M. Trace extracellular but substantial intracellular levels of the glucuronide derivative of AMT (3'-amino-3'-deoxy-5'-β-D-glucopyranosylthymidine, GAMT) were also detected. Moreover, the extent of the formation of AMT, GAZT and ddI-M from the dimer was markedly lowered compared to that with AZT and ddI alone by the hepatocytes. Using hepatocytes in primary culture obtained from rat, monkey and human, large Interspecies variations in the metabolism of AZT-P-ddI were observed. While GAZT and ddI-M, metabolites of AZT and ddI respectively, as well as AZT-MP and ddI-MP were detected in the extracellular compartment of all species, AMT and GAMT were only produced by rat and monkey hepatocytes. No such metabolites were formed by human hepatocytes. The metabolic fate of the dimer by human hepatocytes was consistent with in vivo data recently obtained from HIV-infected patient.

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Methylene Blue Viral Photo-inactivation is Associated with Formation of RNA-Protein Crosslinks. R.A. Floyd, J.E. Schneider, Jr., X.-L. Lin, P. Marble, T. Thomas, Q. Pye, J. Tang. Oklahoma Medical Research Foundation, Oklahoma City, OK. USA

Methylene Blue plus Light (MB+L) is known to inactivate some viruses very efficiently, and is currently in use as a treatment of blood products in order to inactivate undetected viruses, including the human immunodeficiency virus (HIV-1). MB+L forms 8-hydroxyguanine (8-OHGua) in RNA and DNA and protein crosslinks in the RNA bacteriophage Qß. RNA-protein crosslinks occur at a rate that makes them a candidate for the primary inactivation lesion(s) in Qß during MB+L exposure. We show with an infectious RNA assay that Qß RNA is much more rapidly inactivated by MB+L when it is exposed to the intact virion, as compared to purified RNA. This is interpreted to mean that the association of the phage proteins with the genomic RNA of the phage enhance the inactivation of the RNA. In contrast with the Qß phage system, the HIV-1 RNA virus has many proteins in its virion structure, some of which have enzymatic activities. We show that HIV-1 viruses are inactivated by MB+L exposure at a much faster rate than is the reverse transcriptase (RT) associated with the HIV-1 virion. Data collected also suggests that protein-RNA crosslinks form in photo-inactivated HIV-1. Whether the crosslinks are the primary inactivation lesions has yet to be proven. This work is supported by grant number NIH HL 53585.

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DRUG DELIVERY VIA AMINO ACID PHOSPHORAMIDATES

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Nucleosides have become an important class of agents for both viral and cancer chemotherapy. For example, AZT is currently used as an anti-HIV treatment, while Ara-C is used as an anti-tumor agent. Because the activity of these drugs is associated with their ability to be phosphorylated intracellularly, a key mechanism of resistance to these agents has been to reduce the efficacy and levels of nucleoside kinases. Consequently, our laboratory has begun a detailed analysis of the feasibility of using hydrophobic amino acid phorphoramidate prodrugs of antiviral and antitumor nucleosides. Our initial goal was to improve the efficacy of antiviral and antitumor nucleosides. Synthetic methodologies have been developed for the construction of phosphoramidate amino acid nucleosides and biological and biochemical evaluation conducted. Several of the phosphoramidates have demonstrated greater potency than the parent nucleosides, but with little or no associated cytotoxicity in cultures of human cells and in animals. In addition, the compounds were shown to be water soluble and stable indefinitely in human blood. Mechanistic studies attempting to characterize the activity of these unique compounds have revealed for the first time that the metabolism of phospho-monoester amidates of nucleosides in proliferating tissue can proceed through direct P-N bond cleavage by an unknown phosphoramidate hydrolyzing enzyme. Interestingly, these compounds may have differential effects on either viral replication or tumor growth, since we have shown that a subset of biologically active phosphoramidates function as inhibitors and not substrates for this hydrolase activity. Currently, we are attempting to characterize this unique activity in order to determine; 1) the potentially unique cellular function of this enzyme and associated enzymes and 2) the molecular constraints on substrate and inhibitor specificity.

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Identification and Characterization of Topical Microbicides: Inhibitors of HIV Sexual Transmission

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We have developed a series of microtiter-based, high-throughput assays to evaluate the ability of anti-HIV compounds to be used as topical microbicides. These assays include evaluation of efficacy and toxicity in CD4(-) cervical epithelial cells (ME180), fresh human peripheral blood leukocytes and monocyte-macrophages and fresh human dendritic cells from fetal liver. Evaluations were performed using low passage, clinical virus isolates, including isolates representative of the various HIV-1 clades found worldwide, as well as with HIV-2 and SIV. Mechanistic assays (attachment, fusion, RT, integrase, protease) were performed to determine the mode of action of the active topical microbicide. Finally, the toxicity of the topical microbicide to organisms representative of the normal vaginal flora and the activity of the microbicide in the presence of polysaccharides similar to those of the vaginal mucosa were evaluated. A variety of compounds representing diverse anti-HIV mechanisms of action have been studied in our assays to evaluate their ability to be used topically. These studies will be useful for the identification of topical microbicides, which might be used by women prior to sexual