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Effect of Fialuridine (FIAU) on the In vitro Replication of Mitochondrial DNA in Human T Cells and in Human Hepatoblastoma Cells

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Fialuridine (FIAU) is a nucleoside analog which is a potent inhibitor of HBV replication in vitro and in the woodchuck model of chronic HBV infection. The toxicity of FIAU may be related to its inhibition of mitochondrial (mt) DNA replication. Accordingly, the effect of FIAU on mtDNA replication in vitro was investigated. Human T cells (CCRF-CEM) were incubated for 6 days in up to 20 μM FIAU. Total cellular DNA was isolated, normalized for cell number, and slot-hybridized to a $^{32}$P-labeled probe specific for mtDNA sequences. No dose-dependent decrease in the amount of mtDNA was detected. In a control experiment, dideoxycytidine (ddC) inhibited mtDNA replication by 50% at a concentration of approximately 0.2 μM. After 6 days of incubation, both compounds displayed a toxic dose 50% at a concentration of approximately 2 μM. In further experiments, T cells were incubated for 15 days in up to 2.5 μM FIAU, and again, no inhibition of mtDNA was observed. FIAU also failed to inhibit mtDNA replication in either human hepatoblastoma cells (HepG2) or HepG2 cells which constitutively replicate duck HBV. These cells were incubated for 5 days in up to 200 μM FIAU. In contrast, ddC inhibited mtDNA replication in these cells with an $IC_{50}$ between 0.2 and 0.8 μM. An increase in lactic acid production by cultured cells has been correlated to inhibition of mtDNA replication. Treatment of cells with either FIAU or ddC resulted in a dose-dependent and time-dependent increase in lactate levels in the cell medium. Taken together, these data indicate that FIAU may have a negative effect on mitochondria which is not reflected by absolute mtDNA levels. Experiments are in progress to determine whether FIAU induces qualitative alterations in mtDNA, such as deletions, degradation, or point mutations resulting in altered gene expression.

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**A Rapid and High Capacity *In Vitro* Antiviral Assay for Hepatitis B Virus Using Quantitative Polymerase Chain Reaction (PCR).** R. M. Lloyd Jr., J. T. Huong, A. McMillan, and R. F. Schinazi.* Veterans Affairs Medical Center and Department of Pediatrics, Emory University School of Medicine, Atlanta, GA 30033, USA.

Several high capacity *in vitro* assays for discovering novel antiviral drugs against HBV have been developed. However, these methods usually entail tedious and expensive methodologies, using for example, antibody and streptavidin techniques. Our new antiviral assay was developed in order to simplify drug assessment using a 96 well plate format and PCR quantification. For example, 2',3'-dideoxy-5-fluoro-3'-thiacytidine [(-)-FTC] was evaluated in HBV transfected liver cells (2.2.15) after *only 4 days in culture without medium change.* Untreated infected cells PCR product and a standard amount of viral DNA were included in every assay in order to determine the amount of free viral particle produced in the supernatant. The positive virus control, consisting of the PCR product for the culture without drug treatment, was compared to the drug treated product for quantification. The *integrated volume* (IV) values of all PCR products were measured using a phosphorimager or a densitometer after separation of the product on agarose gel. By comparing the drug treated lanes to the untreated lanes, the percent inhibition for the drug was determined. The IV of the negative control compared to the IV for the standard provided a value for the amount of free virus in the supernatant. This was then used to determine the amount of free viral DNA in the culture supernatant. The IV for the negative control cultures containing no drugs from the PCR products was 497.4. The median effective concentration ($EC_{50}$) value for (-)-FTC in 2.2.15 cells, determined from the dose-response curve, was 0.05 μM. This value is similar to the value obtained by a standard 9-14 days assay. The use of PCR for the detection of HBV has many advantages, in particular PCR can generate a signal that allows the detection of low amounts of free virion in the supernatant *(Supported in part by the VA).*