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Effect of Antiviral Nucleoside Analogs on Human Polymerases and Mitochondrial DNA Synthesis. J. L. Martin, C. E. Brown, and J. E. Reardon* Divisions of Virology and Experimental Therapy*. Burroughs Wellcome Co. RTP, NC 27709 USA.

It has been suggested that toxicities observed in HIV- and/or HBV-infected patients treated with antiviral nucleoside analogs may be due to a reduction in mitochondrial DNA synthesis and that this reduction may be due to the inhibition of polymerase gamma. Human polymerases alpha, beta, gamma, and epsilon were purified and the K_i/K_m values were determined for 17 nucleotide analogs with each enzyme under identical conditions of ionic strength, pH, divalent metal ion concentration, and DNA substrate. The inhibition of mitochondrial DNA synthesis was also assessed in an *in vitro* cell culture assay, wherein, the ratio of mitochondrial to cellular DNA was determined after prolonged exposure of cells to clinically relevant concentrations of drug. Treatment of Molt-4 cells with various concentrations of ddC resulted in a reduction of the mitochondrial DNA content of the cells. After 5 days treatment with 0.05 μM ddC, there was an 80% reduction in the ratio of mitochondrial to cellular DNA; however, there was no apparent increase in doubling time of the treated cells. The K_i/K_m value for polymerase gamma with ddCTP/dCTP was 0.088. After 7 days treatment with 0.05 μM FLT, there was a 59% reduction in the ratio of mitochondrial to cellular DNA. At 5 μM, both compounds, at the respective time points, resulted in cell death. In contrast, FIAU caused no reduction in the ratio of mitochondrial to cellular DNA after 7 days exposure to 0.1 or 0.5 μM drug; however, significant cell death was noted after exposure to 5 μM FIAU for 2 days. AZT, at 0.5 or 5.0 μM, caused no significant decrease in the ratio of mitochondrial to cellular DNA after 9 days exposure. The K_i/K_m value for polymerase gamma with AZTTP /dTTP was 51. The 2 experimental compounds, FTC and 935U83, did not reduce the ratio of mitochondrial to cellular DNA at 5 μM or 100 μM, respectively. The K_i/K_m values for polymerase gamma with FTCTP/dCTP and 935U83TP /dTTPwere 35 and 0.40, respectively. Finally, cell death was not noted in AZT-, FTC-, or 935U83-treated cells. These data suggest that inhibition of purified mitochondrial DNA polymerase gamma is not an accurate predictor of potential *in vitro* toxicity of antiviral nucleoside analogs. At this time, it is also not clear whether inhibition of mitochondrial DNA synthesis will be an accurate predictor of potential *in vivo* toxicity of antiviral nucleoside analogs.

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Mixed Infection of Lymphoid Cells, Induced with Epstain-Barr Virus, Adenoviruses, HIV, HTLV-I as a Model for Screening Antivirus Substances

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A problem of virus-associated infections is complex and important one, especially if one of the associants is HIV since among viruses there may occure the interaction by a type of reciprocal strengthening, inhibition, or viruses reproduce independently. A model of the mixed infection of lymphoblastoid cells of phenotypes B and T (Raji, B-95-8, Jurkat, SEM, MT-2) by HIV, sometimes HTLV-I, EBV and Ad was constructed. In some cases a method of cell-producer cocultivation was used. For indication of viruses PCR, molecular DNA-DNA hybridization, ELISA and virological methods have been chosen. To confirm the phenomenon of the mixed infection electron microscopy was used. The capacity of adenovirus type 2 to reproduce in all lines tested was demonstrated though the level of the reproduction level varies. The use of the constructed model of the mixed infection is important in a search of effective antivirus substances as the possibility arises to analyze the influence of the substances on each of virus-associants and their complex, real for conditions of macroorganism.