Oral Session VII

Pharmacology and Clinical Studies

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Influence of template primary structure on 3'-azido-3'-deoxythymidine triphosphate (AZT-TP) incorporation into genomic DNA. Bridges, E.G., LeBoeuf, R.B., Weidner, D.A. and Sommadossi, J.P. Univ. of Alabama at B'ham, B'ham, AL 35294.

We recently reported that 3'-azido-3'-deoxythymidine (AZT) selectively inhibits hemoglobin production and globin gene transcription in differentiating K-562 cells, while 2',3'-dideoxycytidine (ddC) had no effect on the hemoglobin machinery (Mol. Pharmacol., (1990) 38:797-804). This study demonstrated that inhibition of hemoglobin production is not a general effect of dideoxynucleosides in these cells and suggests that template sequence of genes may play a critical role in the observed effects. In the present study, templates containing a specific segment of the gamma-globin gene were constructed and incorporation of AZT-TP, 2',3'-dideoxythymidine-5'-triphosphate (ddTTP) or 2',3'-dideoxycytidine-5'-triphosphate (ddCTP) in these templates was compared to that observed in M13 bacteriophage DNA. Sequencing gel analysis demonstrated that T7 DNA polymerase incorporated AZT-TP into T sites of elongating DNA strands. However, chain termination at noncomplementary G sites was also observed with AZT-TP using the globin template. Such effects were not detected with ddTTP. These misincorporation effects of AZT were demonstrated to be sequence specific. Investigations on the intrinsic fidelity of T7 DNA polymerase in reactions with AZT-TP and without deoxythymidine-5'-triphosphate, resulted in DNA synthesis beyond the first T site, indicating that other normal deoxynucleotides misincorporated at these T sites. Of importance, misincorporation/termination patterns by T7 DNA polymerase in the presence of AZT-TP were not detected by using a M13 template DNA. Conversely, in the absence of competing deoxycytidine-5'-triphosphate, reactions with ddCTP terminated chain elongation at the first C site and no extension products were detected beyond this site. These data demonstrate the importance of using genomic DNA sequences to adequately evaluate the molecular mechanisms by which dideoxynucleosides incorporate into host DNA. The misincorporation of normal deoxy- and dideoxynucleotide substrates in a template sequence-specific manner may be responsible in part for alteration of globin gene expression observed in K-562 cells exposed to AZT. Lastly, the present study raises the question whether sequence-specific misincorporation by host cell polymerases in the presence of AZT may occur within the integrated viral genome, potentially leading to the generation of a mutant viral genotype following extension of mismatched termini. (Supported by NIH grants HL-42125 & AI-25784)