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Inhibition of the 3' to 5' exonuclease activity of mammalian DNA polymerase δ by 3'-azido-3'-deoxythymidine 5'-monophosphate (AZTMP). E.G. Bridges*, A.G. So#, C.K. Tan#, and J.P. Sommadossi*. *Dept. of Pharmacology, Univ. of Alabama at Birmingham, B'ham AL; #Department of Medicine, Univ. of Miami School of Medicine, Miami, FL , USA.

Naturally occurring ribo- and deoxyribonucleoside 5'-monophosphates have been observed to inhibit the 3' to 5' exonuclease activity of mammalian DNA polymerase δ with no significant specificity associated with the base or sugar moiety. Because of the nonspecific inhibition of exonuclease activity by nucleoside 5'-monophosphates and the fact that AZTMP accumulates to millimolar levels intracellularly, the present study was undertaken to determine the effects of AZTMP on the 3' to 5' exonuclease activity of DNA polymerase δ. This activity was sensitive to inhibition by micromolar concentrations of AZTMP, being inhibited by approximately 30% and 80% at 100 μM and 250 μM, respectively. Naturally occurring thymidine 5'-monophosphate slightly inhibited exonuclease activity at 250 μM. Other 2',3'-dideoxythymidine analogs, including the 5'-monophosphate of 3'-amino-3'-deoxythymidine (AMT), a highly toxic in vivo catabolite of AZT, were also assayed for their inhibitory effect on 3' to 5' exonuclease activity and data will be discussed. The inhibition of the 3' to 5' exonuclease activity of DNA polymerase δ by AZTMP and other related metabolites may account in part for the host cell toxicity associated with AZT. The present study suggests that the observed inhibition of RNase H activity of HIV reverse transcriptase by AZTMP is probably mediated through the inhibition by the AZT metabolite on the 3' to 5' exonuclease activity of RNase H. In addition, this data raises the question whether AZTMP inhibition of this activity may lead to potential carcinogenic effects by inhibiting 3' to 5' exonuclease mediated proofreading functions involved in host DNA replication.

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Examination of Toxicity of DDC, DDI, and D4T as Related to Their Effects on Peripheral Neurons Using a Rat PC-12 Pheochromocytoma cell line as model. J.P Sommadossi, and M.Y. Xie; Dept. Pharmacology Univ. of Alabama at Birmingham, B'ham, AL, USA.

With the exception of AZT, 2',3'-dideoxynucleosides (ddNs) evaluated in patients (including DDC, DDI and D4T), have been associated with a painful peripheral neuropathy. In the present study, we evaluated whether a rat PC-12 cell line could be a relevant model for ddN-induced peripheral neuropathy. Effects of ddNs were initially assessed in suspension cultures. Under these conditions, DDC at 25 μM completely inhibited cell proliferation, while no substantial effects were observed at 1 and 10 μM. In contrast, AZT had no inhibitory effects on the proliferation of these cells even after exposure for 14 days at 25 μM. In a similar fashion, D4T and DDI, at concentrations comprised between 1 and 25 μM, respectively, did not substantially inhibit cell growth of PC-12 cells. These experiments were carried out to determine the ddNs' cytotoxic concentrations in order to assess their effects on neurite outgrowth at non-toxic concentrations. PC-12 cells were primed with 50 ng/ml of Nerve Growth Factor (NGF) for 7 days. Cells were then replated on poly-L-lysine coated dishes in the presence, or absence, of NGF, and ddNs studied at concentrations between 0.1 and 25 μM for 7 days. Neurite formation was then scored. AZT, at concentrations up to 25 μM, did not alter the ability of NGF to promote neurite regeneration. In contrast, both DDC, DDI and D4T did affect, in a dose-dependent manner, NGF-promoted neurite outgrowth. DDC and DDI were the most toxic compounds with IC_{50} values of approximately 5 μM. D4T was slightly less toxic with an IC_{50} value of 15 μM for inhibition of neurite regeneration of PC-12 cells. The effects of ddNs on mitochondrial DNA synthesis and other neural molecular targets will be discussed. These data suggest that differentiated PC-12 cells represent a relevant system for ddN-induced peripheral neuropathy and can be used to elucidate the cellular and molecular mechanism(s) responsible for the observed effects.