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ZIDOVUDINE INHIBITS THYMIDINE PHOSPHORYLATION: A NOVEL SITE OF POTENTIAL TOXICITY IN NON-MYTOTIC CELLS

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Zidovudine (3'-azido-3'-deoxythymidine, AZT) has been a staple of highly active antiretroviral therapy (HAART) for many years. Unfortunately, long-term use of AZT is also associated with various tissue toxicities, including hepatotoxicity and cardiomyopathy, associated with mitochondrial DNA depletion. The triphosphate form of AZT (AZT-TP) is a known inhibitor of the mitochondrial polymerase γ and has been targeted as the source of the mitochondrial DNA depletion. Previous work in this laboratory with isolated rat heart mitochondria in 2–3 h experiments suggests that AZT is not phosphorylated beyond the monophosphate (AZT-MP), which accumulates with no detection of AZT-TP. Rather, AZT was shown to be a much more potent inhibitor of thymidine phosphorylation ($7.0 \pm 1.0 \mu\text{M}$) than AZT-TP is of polymerase γ ($>100 \mu\text{M}$), suggesting that depletion of mitochondrial stores of TTP may limit replication. The purpose of this work was to investigate the hypothesis that an identical mechanism might account for the hepatotoxicity seen with long-term use of AZT. Isolated rat liver mitochondria were incubated with labelled thymidine or AZT, and the rate and extent of phosphorylation to nucleotides was determined by HPLC analysis of acid soluble extracts of the incubated mitochondria. The results obtained showed that AZT is phosphorylated only to AZT-MP and that AZT inhibited the production of TTP, with an IC_{50} of $8.9 \pm 1.8 \mu\text{M}$ AZT. This is comparable with the results seen in isolated rat heart mitochondria. The kinetics of thymidine phosphorylation to TMP differs between heart and liver. The maximal velocity (V_{max}) and affinity constant (K_{m}) are an order of magnitude higher in liver than in heart. Liver also displays either strong negative cooperativity or the presence of two enzymes, one with a V_{max} and K_{m} similar to TK2 in rat heart mitochondria and one with much higher V_{max} and K_{m} . This second enzyme may represent several possibilities

including contaminating thymidine kinase 1, a different isoform of TK2, or another yet uncharacterized kinase.

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31

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