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

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Zidovudine (3'-azido-3'-deoxythymidine, AZT) is a thymidine analogue pro-drug that can be phosphorylated by host cell enzymes to the triphosphate, which is a potent inhibitor of the viral reverse transcriptase. AZT has been a staple of highly active antiretroviral therapy (HAART) for many years. In the early days of AIDS therapy, AZT was given alone at relatively high concentrations and its long-term use was associated with various tissue toxicities, including hepatotoxicity and cardiomyopathy, associated with mitochondrial DNA depletion. In more recent therapy, AZT in combination with other nucleoside analogues is associated with a lipodystrophy that may be of mitochondrial origin. The triphosphate form of AZT (AZT-TP) is a known inhibitor of the mitochondrial polymerase γ and has been targeted as the potential source of the mitochondrial DNA depletion. This laboratory's previous work with isolated rat heart mitochondria in short-term experiments suggested that AZT was not phosphorylated beyond the monophosphate (AZT-MP). AZT-MP accumulated in the matrix and AZT-DP and AZT-TP were not detected. Instead, we found that AZT was a potent inhibitor of thymidine phosphorylation ($7.0 \pm 1.0 \mu\text{M}$) acting at the level of the mitochondrial thymidine kinase (TK2). In non-mitotic tissues such as cardiomyocytes, cytoplasmic thymidine kinase (TK1) is not expressed and the cell's supply of TTP is generated solely through TK2. We therefore suggest the novel hypothesis that toxicity of AZT in non-mitotic tissue is caused by the direct inhibition of TK2 limiting the cellular pool of TTP. The low level of TTP limits mitochondrial DNA replication, ultimately leading to mitochondrial dysfunction associated with mitochondrial DNA depletion. This hypothesis was tested in this work using two approaches. In one, an isolated perfused heart system was used to extend our investigation of AZT and thymidine phosphorylation

to the whole cell [Susan-Resiga *et al. Antiviral Therapy* 2004; 9(6):L22 ([Abstract 32](#))] In the second, we sought to extend our observations in isolated heart mitochondria to mitochondria isolated from other tissues, beginning with liver mitochondria [Lynx *et al Antiviral Therapy* 2004; 9(6):L22 ([Abstract 31](#))]. Thymidine was readily phosphorylated to TTP in both of these systems, with TTP the predominant product in the perfused heart and TMP the predominant product in isolated liver mitochondria. However, the phosphorylation of AZT beyond AZT-MP was not detected in either system. The production of TTP and AZT-MP in isolated liver mitochondria was an order of magnitude higher than observed in heart mitochondria. Finally, AZT inhibited thymidine phosphorylation in both systems with IC₅₀s of 8.9 ± 1.8 µM in liver mitochondria and 24 ± 5 µM in the perfused heart.

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