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THYMIDINE AND ZIDOVUDINE METABOLISM IN ISOLATED RAT HEART: ZIDOVUDINE INHIBITION OF THYMIDINE PHOPSPHORYLATION

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Zidovudine (3'-azido-3'-deoxythymidine, AZT) is a thymidine analogue pro-drug that is phosphorylated in the host to AZT triphosphate (TP), which functions by inhibiting viral reverse transcriptase. AZT has been part of the highly active antiretroviral therapy (HAART) for many years; however long-term use of AZT was linked to various tissue toxicities, including cardiomyopathy, and was associated with mitochondrial DNA depletion. So far, the prevailing hypothesis for AZT toxicity has been the inhibition of mitochondrial DNA polymerase γ by AZT-TP. Our previous work with isolated heart mitochondria Cardiovasc Toxicol. (McKee EE *et al*., Cardiovasc Toxicol. 2004;4(2):155-67) demonstrated that AZT was phosphorylated only to the monophosphate (MP) under conditions in which thymidine (T) was readily phosphorylated to the triphosphate. This suggested that the cardiac mitochondrial toxicity observed for this drug was not likely to be caused by AZT-TP inhibition of the mitochondrial replication polymerase. Rather, we demonstrated that AZT was a potent inhibitor of thymidine phosphorylation suggesting that mitochondrial DNA replication was limited by the TTP pool.

The present work extends the above observations to the intact isolated perfused heart and examines whole cell metabolism of thymidine and AZT. Tritiated thymidine is readily converted to labelled TTP in a time and concentration dependent manner in the perfused heart. Steady-state level of the TDP/TTP pool is reached in 60–90 min. The level of labelled TMP is quite low, suggesting that thymidine kinase (TK) is the rate limiting step in phosphorylation. The kinetics of TTP phosphorylation displays negative cooperativity, similar to the observations with cloned and purified mitochondrial TK2 (Wang and Ericsson, 2000). Labeled AZT is converted in a time and concentration dependent manner to AZT-MP, the only phosphorylated form detected even after 3 h of perfusion.

AZT phosphorylation in perfused heart follows simple Michaelis–Menten kinetics, as opposed to thymidine. The presence of AZT in the perfusate inhibits the conversion of labelled thymidine to TTP with an IC_{50} of 24 ±5 mM. These data taken together extend the observations made in isolated heart mitochondria and suggest that mitochondrial toxicity in the heart may be caused by a limiting cellular pool of TTP lowering the rates of mitochondrial DNA replication.

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