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3′-Azido-3′-deoxythymidine (AZT) is a competitive inhibitor of thymidine phosphorylation in isolated rat heart and liver mitochondria

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Abbreviations:
AIC, Akaike's information criterion
AIDS, acquired immunodeficiency syndrome
AZT, 3′-azido-3′-deoxythymidine
AZTMP, 3′-azido-3′-deoxythymidine-5′-monophosphate
AZTTP, 3′-azido-3′-deoxythymidine-5′-triphosphate
IC_{50}, 50% inhibitory concentration
HAART, highly active anti-retroviral therapy
HIV, human immunodeficiency virus
NRTI, nucleoside analog reverse transcriptase inhibitor

ABSTRACT

Long-term use of 3′-azido-3′-deoxythymidine (AZT) is associated with various tissue toxicities, including hepatotoxicity and cardiomyopathy, and with mitochondrial DNA depletion. AZT-5′-triphosphate (AZTTP) is a known inhibitor of the mitochondrial DNA polymerase γ and has been targeted as the source of the mitochondrial DNA depletion. However, in previous work from this laboratory with isolated rat heart and liver mitochondria, AZT itself was shown to be a more potent inhibitor of thymidine phosphorylation (IC_{50} of 7.0 ± 1.0 μM AZT in heart mitochondria and of 14.4 ± 2.6 μM AZT in liver mitochondria) than AZTTP is of polymerase γ (IC_{50} of >100 μM AZTTP), suggesting that depletion of mitochondrial stores of TTP may limit replication and could be the cause of the mitochondrial DNA depletion observed in tissues affected by AZT toxicity. The purpose of this work is to characterize the nature of AZT inhibition of thymidine phosphorylation in isolated rat heart and rat liver mitochondria. In both of these tissues, AZT was found to be a competitive inhibitor of the phosphorylation of thymidine to TMP, catalyzed by thymidine kinase 2. The inhibition constant (K_i) for heart mitochondria is 10.6 ± 4.5 μM AZT, and for liver mitochondria K_i is 14.0 ± 2.5 μM AZT. Since AZT is functioning as a competitive inhibitor, increasing thymidine concentrations may be one mechanism to overcome the inhibition and decrease AZT-related toxicity in these tissues.

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1. Introduction

AZT is an analog of the thymidine deoxynucleoside and is a member of the class called the nucleoside-analog reverse transcriptase inhibitors. AZT and other members of this class function by inhibiting the HIV reverse transcriptase. This halts the life cycle of the virus and slows the progression of AIDS. Originally, AZT was given at a high dosage in monotherapy, and long-term treatment resulted in many tissue toxicities, including myopathies, dilated cardiomyopathy, and hepatotoxicity [1–11]. When AZT was withdrawn from the patient's therapy regimen, these toxicities would resolve, suggesting that they are due to AZT and are not symptoms of AIDS [6,8]. The modern HAART regimen uses lower dosage AZT in combination with other drugs. This has caused these toxicities to become rare today but has lead to new problems related to AZT therapy, including lipodystrophy and hematological toxicities [12,13].

Mitochondrial damage due to mitochondrial DNA depletion is believed to be the cause of these toxicities [14–16]. The current prevailing hypothesis for NRTI toxicity is that the NRTI-triphosphate is inhibiting the mitochondrial DNA polymerase γ [17]. However, this mechanism seems less likely for AZT which is not readily phosphorylated beyond AZTMP since it is a poor substrate for thymidylate kinase [18,19]. As a result of this, AZTTP has never been detected at a concentration high enough to inhibit polymerase γ (IC_{50} of >100 μM) [16,18,20].

Previous work from this laboratory has suggested an alternative mechanism in which AZT inhibits the phosphorylation of thymidine. Trials in isolated rat heart and liver mitochondria carried out at 1 μM thymidine, the approximate physiological concentration [21], revealed an IC_{50} of 7.0 ± 1.0 μM AZT for heart mitochondria and 14.4 ± 2.6 μM AZT for liver mitochondria [22,23]. These IC_{50} values reflect inhibitory concentrations of AZT, not AZTTP, and are considerably lower than the concentration of AZTTP needed to inhibit polymerase γ. The steady-state 1.5 h post-dose peak serum concentration seen with chronic oral administration of 250 mg AZT every 4 h is 2.32 μM with a range of 0.19–5.46 μM [24]. This dosage is slightly higher than the recommended dosage of oral AZT of 200 mg every 4 h used in monotherapy; however, both of these dosages are in the range of dose-independent kinetics. The observed IC_{50} data from heart and liver mitochondria is close to these serum concentrations and suggests that AZT inhibition of thymidine phosphorylation could play a role in the clinical toxicity observed in AZT monotherapy. In the present day HAART regimen, AZT is dosed at 600 mg total per day (either 200 mg three times per day or 300 mg twice per day), resulting in a steady-state serum AZT concentration of ~0.8 μM [25]. This lower serum concentration may explain why the toxicities associated with the higher dosage monotherapy have become rare in current regimens.

Non-mitotic tissues, like heart and liver, may depend solely on thymidine kinase 2 to salvage thymidine and maintain the intracellular TTP pool. Given this inhibition, it is possible that in these tissues AZT inhibition of thymidine phosphorylation is depleting the TTP pool, which slows mitochondrial DNA replication and could lead to the observed mitochondrial DNA depletion associated with AZT toxicity. Since AZT and thymidine both are phosphorylated by the same enzyme, thymidine kinase 2, in isolated heart and liver mitochondria, it is likely that the observed inhibition is due to AZT directly competing with thymidine for phosphorylation by thymidine kinase 2. In this work, we hypothesize that AZT inhibition of thymidine phosphorylation best fits the model of competitive inhibition.

2. Materials and methods

2.1. Isolation and incubation of rat heart and liver mitochondria

Mitochondria were isolated from Harlan–Sprague–Dawley rat heart and liver using the methods described previously [22,23]. The isolated mitochondria were then incubated at 30 °C in a medium defined previously [22,23]. Concentrations and specific radioactivities of [methyl-^{3}H]-thymidine used in these incubations is noted in the figure legends.

2.2. Detection of mitochondrial phosphorylation of thymidine and AZT by direct precipitation

A 0.2 mL aliquot of the incubation medium was removed at various time points during incubation and was mixed with an equal volume of 10% trichloroacetic acid. This mixture was kept on ice for at least 10 min and then centrifuged. 0.35 mL of the acid-soluble supernatant were removed and neutralized with 350 mg of resin (AG-11A8) and 0.21 mL of water. The neutralized extract was filtered and analyzed by HPLC as described below. This method yields the total of the nucleoside and nucleotide components found in the medium and in the acid-soluble portion of the mitochondrial matrix and does not differentiate between phosphorylation within the matrix and phosphorylation outside of the matrix. However, previous work has demonstrated that phosphorylation is a matrix event [22].

2.3. HPLC analysis

[Methyl-^{3}H]-thymidine and its phosphorylated intermediates from the neutralized acid-soluble extract described in the previous section were identified and quantitated using reverse-phase HPLC with an Alltech absorbosphere nucleoside/nucleotide column connected to an in-line UV monitor (254 nm) and a Radiomatic flow-through scintillation counter using methods described previously [22].

2.4. Data treatment

Data was analyzed with Enzyme Kinetics Pro v2.36 to obtain values for AIC and K_i ± standard deviation. Graphs were generated with Sigma Plot 9.01.

3. Results

3.1. Time course of thymidine phosphorylation

Prior work from this laboratory has shown that thymidine phosphorylation in isolated rat heart and liver mitochondria is