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Nucleoside reverse transcriptase inhibitors (NRTIs)-induced expression profile of mitochondria-related genes in the mouse liver

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Abstract

Mitochondrial dysfunction has been implicated in the adverse effects of nucleoside reverse transcriptase inhibitors (NRTIs) used to treat HIV-1 infections. To gain insight into the mechanism by which NRTIs alter mitochondrial function, the expression level of 542 genes associated with mitochondrial structure and functions was determined in the livers of p53 haplodeficient (+/−) C3B6F$_1$ female mouse pups using mouse mitochondria-specific oligonucleotide microarray. The pups were transplacentally exposed to zidovudine (AZT) at 240 mg/kg bw/day or a combination of AZT and lamivudine (3TC) at 160 and 100 mg/kg bw/day, respectively, from gestation day 12 through 18, followed by continuous treatment by oral administration from postnatal day 1–28. In addition, AZT/3TC effect was investigated in wild-type (+/+) C3B6F$_1$ female mice. The genotype did not significantly affect the gene expression profile induced by AZT/3TC treatment. However, the transcriptional level of several genes associated with oxidative phosphorylation, mitochondrial tRNAs, fatty acid oxidation, steroid biosynthesis, and a few transport proteins were significantly altered in pups treated with AZT and AZT/3TC compared to their vehicle counterparts. Interestingly, AZT/3TC altered the expression level of 153 genes with false discovery rate of less than 0.05, in contrast to only 20 genes by AZT alone. These results suggest that NRTI-related effect on expression level of genes associated with mitochondrial functions was much greater in response to AZT/3TC combination treatment than AZT alone.

Keywords: Mitochondria; Mouse MitoChip; Microarray; Nucleoside reverse transcriptase inhibitors; p53 haplodeficient (+/−) C3B6F$_1$ female mouse; p53 wild-type (+/+) C3B6F$_1$ female mouse

1. Introduction

The use of highly active antiretroviral therapy (HAART) combination regimen has resulted in a sharp decline in AIDS-related morbidity and mortality (Hogg et al., 1999; Palella et al., 1998, 2006). Among different classes of antiretroviral drugs that comprise the HAART regimen, nucleoside reverse transcriptase inhibitors (NRTIs) remain the cornerstone of combination therapy (Squires, 2001). A dual NRTI combination of zidovudine (AZT) and lamivudine (3TC) is effective in decreasing the viral load in HIV-infected pregnant women (Staszewski et al., 1996; Lambert et al., 2003). AZT alone or in combination with other antiretroviral agents effectively reduces mother-to-child transmission of the virus (Fiscus et al., 1996, 2002). It is evident that NRTIs are potent against HIV-1; however, the efficacy of these drugs can be seriously compromised by a wide range of severe adverse effects that are believed to be due to drug-induced mitochondrial dysfunction (Cherry and Wesselingh, 2003).

Abbreviations: mtDNA, mitochondrial DNA; nDNA, nuclear DNA; NRTIs, nucleoside reverse transcriptase inhibitors.

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The antiretroviral action of NRTIs is due to their active triphosphate forms that can suppress viral replication by inhibiting viral reverse transcriptase and/or can function as DNA chain terminators by incorporation into replicating viral DNA (Lewis et al., 1994; Squires, 2001). Nevertheless, these active metabolites may also serve as weak to modest substrates for different human DNA polymerases, and exhibit the greatest affinity for DNA polymerase gamma which is responsible for the replication and repair of the mitochondrial DNA (mtDNA) (Martin et al., 1994). It is believed that the NRTI-induced inhibition of polymerase gamma results in the depletion of mtDNA leading to mitochondrial dysfunction (Lewis and Dalakas, 1995; Brinkman et al., 1998). Supporting this notion are studies that demonstrated a decrease in mtDNA content in skeletal and cardiac muscle of fetal monkeys transplacentally exposed to AZT and AZT/3TC (Gerschenson and Poirier, 2000; Gerschenson et al., 2004). Lower mtDNA levels have also been reported in peripheral blood leukocytes of infants perinatally exposed to AZT compared to non-exposed individuals (Poirier et al., 2003). However, a growing body of evidence indicates that defects in mitochondrial respiratory chain complexes caused by oxidative DNA damage, and/or induction of mutations in mtDNA (Barile et al., 1998; de la Asuncion et al., 1999; Lewis et al., 2001; Dagan et al., 2002; Barret et al., 2003) might play an equally important role in enhancing mitochondrial damage during NRTI exposure. In a cohort study conducted in France, it was found that eight infants born to HIV-infected mothers receiving antiretroviral drugs during pregnancy exhibited significant mitochondrial dysfunction in various tissues as a result of altered activities of the respiratory chain complexes (Blanche et al., 1999). In another study, impaired mitochondrial function was attributed to reduced oxidative phosphorylation activity, while mtDNA level was unaltered in the skeletal muscle of uninfected infants perinatally exposed to antiretroviral drugs (Barret et al., 2003). A lack of correlation between inhibition of polymerase gamma and mtDNA depletion has also been illustrated in human molt-4 cells exposed to different nucleoside analogues *in vitro* (Martin et al., 1994).

Clinical and laboratory findings suggest mitochondrial toxicity as one of the key factors associated with many of the NRTI-related severe adverse effects including, myopathy, pancreatitis, neuropathy, hepatic steatosis and lactic acidosis (Montessori et al., 2004). These drug-related toxicities are of great concern, particularly in children who have been exposed to NRTIs *in utero* or neonatally because of the potential impact on growth and the likelihood of a greater cumulative exposure (Vigano and Giacomet, 2005). The potential long-term effects of persistent mitochondrial damage as a consequence of *in utero* exposure to AZT has been well illustrated in female mice that showed a higher number of mutations in mitochondrial tRNAs in cardiac muscle at 18-months of age (Walker et al., 2004).

It is, therefore, critical to understand the molecular basis of mitochondrial dysfunction during perinatal exposure to NRTIs. To address this issue, we evaluated the transcriptional level of 542 mitochondria-related genes in the liver of p53 haplodeficient and wild-type $C3B6F_1$ mice perinatally exposed to AZT alone or in combination (AZT/3TC). Damaged mitochondria are known to trigger apoptosis through the release of proteins, such as cytochrome *c* and activated caspase. However, the release of proteins is facilitated by p53-mediated translocation of *Bax* protein to the mitochondria (Schuler et al., 2003). In view of this, a lack of one functional copy of the p53 gene in the $C3B6F_1$*trp*53 (+/−) haplodeficient mouse would be expected to partially inhibit translocation of Bax to the mitochondria and thus, apoptosis. This would result in more cells with damaged mitochondria surviving in p53 haplodeficient (+/−) mice compared to the wild-type counterpart. Therefore, in this study, the $C3B6F_1$*trp*53 (+/−) haplodeficient mouse model was used assuming that NRTI-induced altered mitochondrial function could be detected earlier than in wild-type animals. Here, we report microarray analysis that indicated that NRTI-induced mitochondrial dysfunction may not be limited to the inhibition of DNA polymerase gamma, but rather to the functionality of other mitochondrial targets which may have been affected by the drug. These results could have significant clinical implications in the planning of new treatment strategies towards reducing the severity of drug-related adverse effects in children born to HIV-infected mothers.

2. Materials and methods

2.1. Animal husbandry

Animals used in this study were part of an ongoing National Toxicology Program (NTP) project at the National Center for Toxicological Research (NCTR). The project is investigating the mechanism of toxicity and carcinogenicity of perinatal exposure of AZT alone and in combination with 3TC in the $C3B6F_1$*trp*53 (+/−) haplodeficient transgenic mouse model. The $C3B6F_1$*trp*53(+/−) mice were produced by mating Taconic C57BL/6(N5)*trp*53(−/−) males with C3H/HeNTac females, and $C3B6F_1$*trp*53(+/+) mice were produced by mating C3H/HeNTac females with Taconic C57BL/6(N5)*trp*53(+/+) males. Mice were raised in a pathogen-free environment at NCTR and treated according to the Institutional Animal Care and Use Committee guidelines. Mice were housed in standard polycarbonate cages with hardwood chip bedding and maintained at 23 °C with a relative humidity of 50%. Animals were conditioned to a 12-h light/12-h dark cycle with lights on from 06:00 to 18:00 h daily and were given NIH-31 diet and filtered tap water provided *ad libitum*. Animals were weighed and monitored daily for abnormal clinical signs.