

■ **VIROLOGY****Cardiac Mitochondrial Compromise in 1-Year-Old *Erythrocebus patas* Monkeys Perinatally Exposed to Nucleoside Reverse Transcriptase Inhibitors**

Divi RL, Leonard SL, Kuo MM, Walker BL, Orozco CC, St Claire MC, Nagashima K, Harbaugh SW, Harbaugh JW, Thamire C, Sable C, and Poirier MC. Cardiac mitochondrial compromise in 1-yr-old *Erythrocebus patas* monkeys perinatally-exposed to nucleoside reverse transcriptase inhibitors. *Cardiovasc Toxicol* 5: 333–46, 2005.

Given the current, rapid spread worldwide of HIV-1 among young women of childbearing age, and the effectiveness of antiretroviral drugs to protect the infant from maternal-fetal viral transmission, the number of HIV-1–uninfected children exposed perinatally to highly active antiretroviral therapy (HAART) is likely to increase rapidly. HAART typically includes two nucleoside reverse transcriptase inhibitors (NRTIs), examples of which include zidovudine (AZT), lamivudine (3TC), didanosine (ddI), and stavudine (d4T). These are “dideoxy-type” nucleoside analogs that become phosphorylated and incorporated into viral and host DNA, terminating DNA replication and inducing mutagenesis. Mitochondria are potential sites of toxicity, as NRTIs become incorporated into mitochondrial DNA (mtDNA) and specifically inhibit mitochondrial polymerase γ , causing mtDNA depletion.

Clinical manifestations of mitochondrial toxicity include fatigue, lactic acidosis, cardiac and skeletal muscle myopathy, and abnormal results on an echocardiogram. Morphologically damaged mitochondria, mitochondria with altered oxidative phosphorylation (OXPHOS) capacity, and changes in mtDNA quantity have been found in patients with these signs and symptoms. In HIV-1–uninfected infants exposed to NRTIs, clinical evidence of mitochondrial dysfunction is infrequent. However, two HIV-1–uninfected children, born to mothers receiving AZT and 3TC during pregnancy, died at about 1 year of age from severe persistent mitochondrial toxicity, and symptoms of mitochondrial dysfunction have been found in 26 children from a cohort of 2,644 NRTI-exposed children (Barret B et al. *AIDS* 17: 1769–85, 2003). Furthermore, evidence of mitochondrial morphological damage, revealed by electron microscopy (EM) and mtDNA depletion, has been reported in a large fraction of clinically normal infants born to HIV-1–infected women receiving NRTI therapy during pregnancy (Divi RL et al. *AIDS* 18: 1013–21, 2004). The mtDNA depletion appears to persist for up to 2 years of age (Poirier MC et al. *J Acquir Immune Defic Syndr* 33: 175–83, 2003). It is therefore important to evaluate the potential for NRTI-induced mitochondrial toxicity in

these children and relevant animal models.

We investigated cardiac mitochondrial integrity in 1-year-old *Erythrocebus patas* monkeys exposed perinatally to human-equivalent NRTI dosing protocols. The patas monkey model has the advantage that its drug pharmacokinetics are similar to those in humans, and drug effects can be examined in the absence of confounding viral infection. To mimic human clinical exposures, the NRTIs were given to both the dam for the last half of gestation and to the newborn for the first 6 weeks of life. Exposures included no drug (n = 4), AZT (n = 4), AZT/3TC (n = 4), AZT/ddI (n = 4), and d4T/3TC (n = 4). The study included assessments of maternal and infant clinical chemistry and cardiac function by echocardiogram before and after parturition. Heart tissue, taken from 1-year-old offspring, was examined for mitochondrial morphology (via EM), mtDNA quantitation, and OXPHOS enzyme-specific activities.

Functionally, no difference was observed between unexposed and NRTI-exposed patas infants, as they all behaved similarly and had normal echocardiogram results. In addition, OXPHOS enzyme activities were similar in heart mitochondria from all groups. However, EM studies revealed significant mitochondrial morphological damage in hearts from NRTI-exposed animals compared with unexposed animals ($P < .05$) (Figure 1). We found mitochondrial proliferation, swollen and disrupted cardiac myofibrils, visibly misaligned sarcomeres, partial or complete erosion of mitochondrial membranes, and replacement of cristae with clear space (Figure 1, parts B and C). Clusters of highly damaged cardiac mitochondria, suggestive of clonal expansions, were observed in the hearts of all NRTI-exposed patas infants but not in the hearts of unexposed controls (Figure 1, part C). In addition to the mitochondrial morphological damage, levels of mtDNA were elevated in the hearts of all NRTI-exposed monkeys, compared with controls (AZT/ddI > AZT/3TC > AZT > d4T/3TC > control; $P < .05$).

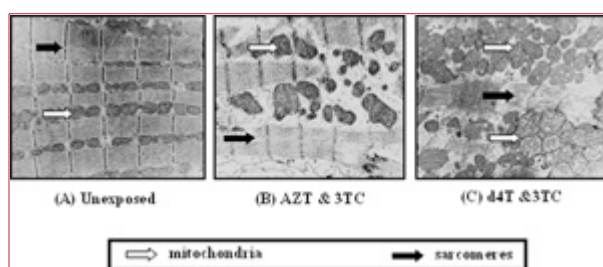


Figure 1. Photomicrographs of *Erythrocebus patas* cardiac tissue at 1 year of age, with no drug exposure (A) and after *in utero* (10 wk) and post-birth (6 wk) exposures to zidovudine (AZT) plus lamivudine (3TC) (B), and stavudine (d4T) plus 3TC (C).

This study showed that, during the first year of life, growing hearts from patas infants exposed perinatally to NRTIs regained the OXPHOS enzyme functionality that was altered in similarly exposed monkeys at birth. However, at 1 year of age, patas monkey hearts showed increases in mtDNA quantity and mitochondrial morphological damage by EM.

Mitochondria with altered morphology were clustered in groups suggestive of clonal expansions of mitochondria that had sustained pathologic mutations and/or deletions. Similar

damage has been found in AZT-exposed aging mice (Walker DM et al. *Cardiovasc Toxicol* 4: 133–53, 2004). This study demonstrates that transplacental NRTI exposures in non-human primates results in persistent cardiac pathology on a molecular level. The data suggest that, in the absence of intervention, cardiac insufficiency may arise later in life in NRTI-exposed patas monkeys and humans.

Rao L. Divi, PhD

Staff Scientist

Laboratory of Cancer Biology and Genetics

divir@exchange.nih.gov

Miriam C. Poirier, PhD

Senior Investigator

Laboratory of Cancer Biology and Genetics

NCI-Bethesda, Bldg.37/Rm. 4032

Tel: 301-402-1835

Fax: 301-402-8230

poirierm@exchange.nih.gov

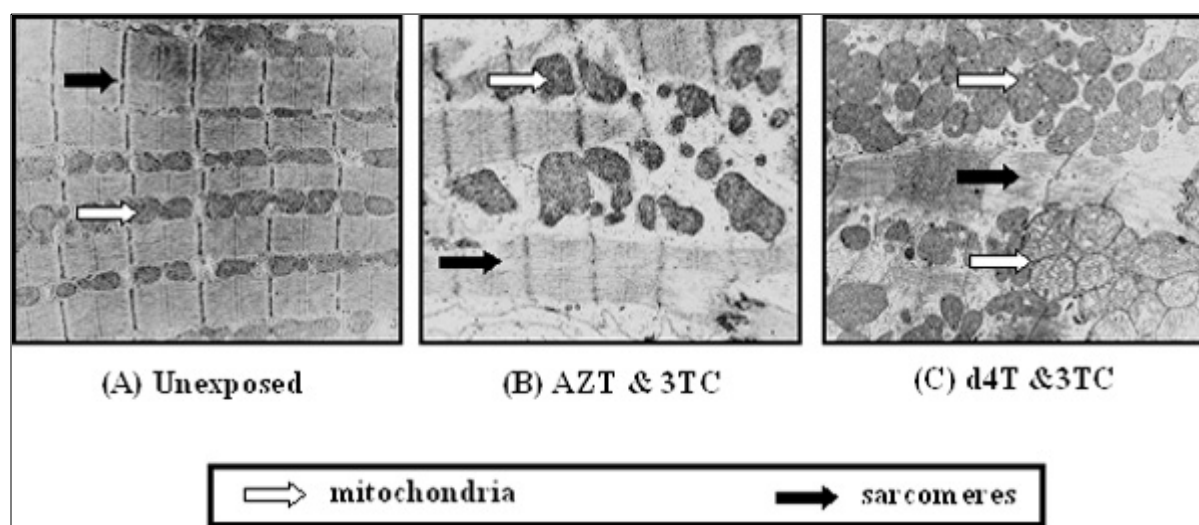


Figure 1. Photomicrographs of *Erythrocebus patas* cardiac tissue at 1 year of age, with no drug exposure (A) and after *in utero* (10 wk) and post-birth (6 wk) exposures to zidovudine (AZT) plus lamivudine (3TC) (B), and stavudine (d4T) plus 3TC (C).