Perspective

Overview of Mitochondrial Toxicity of Nucleoside Reverse Transcriptase Inhibitors

At the International AIDS Society–USA course in New York in October 2001, Marshall J. Glesby, MD, PhD, reviewed potential mechanisms of nucleoside reverse transcriptase inhibitor-associated mitochondrial toxicity and discussed the potential clinical manifestations of mitochondrial dysfunction. For a review of new research on this topic presented at the 2002 Retrovirus Conference, please consult the update on complications of HIV infection and antiretroviral therapy on page 11 of this issue.

Mitochondria provide energy required for normal cell function, and thus tissue function, by producing adenosine triphosphate (ATP) via oxidative phosphorylation. They also regulate a number of other cellular processes, including apoptosis. Mitochondria are present in all cells, except erythrocytes, in numbers of 1 to more than 1000. The number of mitochondria correlates with the degree of cellular and tissue metabolic activity. Mitochondria have their own DNA, likely reflecting a bacterial origin and endocytosis by primitive eukaryotic cells during early evolution.

Mitochondrial DNA (mtDNA) is a small circular DNA species, consisting of approximately 16,000 bases, that codes for 13 polypeptides (as well as transfer and ribosomal RNAs), some of which are key proteins in oxidative phosphorylation. Whereas nuclear DNA (nDNA) is replicated by alpha DNA polymerase (Figure 1A), mtDNA is replicated by a gamma polymerase (Figure 1B), which has a relatively high error rate and some repair capacity. Most mitochondrial proteins are coded by nDNA.

Dr Glesby is Assistant Professor of Medicine and the Co-Director of the Cornell Clinical Trials Unit at Weill Medical College of Cornell University, New York, New York.

Nucleoside reverse transcriptase inhibitors (nRTIs) act via their incorporation into the growing viral DNA chain during reverse transcription, with incorporation resulting in chain termination. The nRTIs also inhibit gamma polymerase (Figure 1C) in vitro, and thus inhibit mtDNA synthesis. Consequent mtDNA depletion (or mutation) can result in insufficient energy production and cell dysfunction and in tissue and organ dysfunction when sufficient numbers of normally functioning mitochondria are not present (Lewis and Dalakas, *Nat Med*, 1995). In addition to the potential effects of gamma polymerase inhibition, nRTIs may also be associated with oxidative damage to mitochondria (de la Asuncion et al, *J Clin Invest*, 1998), inhibition of mitochondrial enzymes (Barile et al, *Biochem Pharmacol*, 1994), uncoupling of the electron transport chain from ATP synthesis, and induction of apoptosis (Kakuda, *Clin Ther*, 2000).

Probable Clinical Manifestations of nRTI-Related Mitochondrial Toxicity

Spurring the identification of a potential association between nRTI treatment and mitochondrial toxicity was the recognition that effects observed in nRTI-treated patients resembled clinical manifestations of inherited mitochondrial diseases. Many of these diseases involve a variety of organs and organ systems, including the brain, peripheral nerves, heart muscle, kidney, liver, endocrine system, gastrointestinal system, and bone marrow. Many of the effects associated or believed to be associated with mitochondrial toxicity, however, are difficult to distinguish from effects associated with HIV infection itself. It is also recognized that nRTIs differ in regard to potential mitochondrial toxicities.

Probable manifestations of mito-

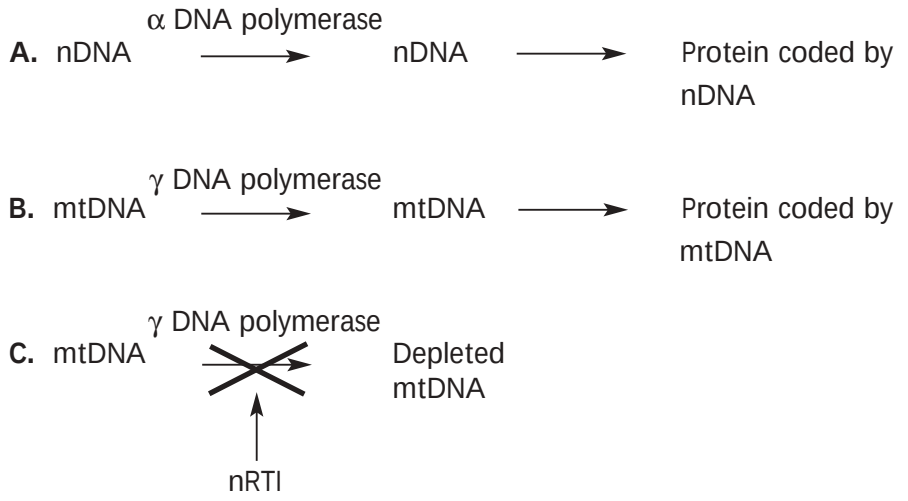

Figure **1.** Replication of nuclear DNA (nDNA) and mitochondrial DNA (mtDNA). In row A, normal nDNA replication in cells by alpha DNA polymerase; in row B, normal mtDNA replication in mitochondria by gamma DNA polymerase; and in row C, the hypothesized inhibition of gamma DNA polymerase by a nucleoside reverse transcriptase inhibitor (nRTI), resulting in the depletion of mtDNA and mtDNA-encoded proteins and consequently mitochondrial dysfunction. Adapted from Brinkman et al, *Lancet*, 1999.

chondrial dysfunction in patients receiving nRTIs include cardiomyopathy, myopathy, peripheral neuropathy, pancreatitis, proximal renal tubular dysfunction, and hepatic steatosis and lactic acidosis.

Cardiomyopathy

HIV infection itself has been demonstrated to cause cardiomyopathy. Cardiac muscle has a large energy demand and thus features a large mitochondria population. Studies to date, although not providing definitive data, implicate zalcitabine, didanosine, and zidovudine in cardiomyopathy; most evidence is in the form of case studies showing reversal of cardiomyopathy when nRTI treatment was stopped. There is some evidence of a cardiotoxic effect of high doses of zidovudine in animals. Autopsy series in non-HIV-infected patients with ischemic heart disease have shown mtDNA damage or mutations in the heart (Corral-Debrinski et al, JAMA, 1991), and it has been suggested that mitochondrial dysfunction might contribute to atherosclerosis in HIV-infected patients (Lewis, *J Mol Cell Cardiol*, 2000).

Myopathy

Both HIV infection and zidovudine treatment have been associated with myopathy, with the respective disorders being difficult to distinguish on a clinical basis. In AIDS Clinical Trials Group (ACTG) study 016, myopathy developed in 1.8% of patients receiving zidovudine monotherapy at 1200 mg per day. Analysis of data from 1067 treatment-naive patients in ACTG 175, in which zidovudine was used at the current dosage of 600 mg per day, showed that only 6 developed myopathy (Simpson et al, AIDS, 1998). Four of these patients received didanosine, 1 received zidovudine/zalcitabine, and 1 received zidovudine/didanosine. There are data indicating that both clinical and tissue abnormalities in myopathy can be reversed when zidovudine treatment is discontinued, and there are some data indicating the presence of mtDNA depletion in zidovudine-associated myopathy.

Peripheral Neuropathy

HIV-associated distal sensory polyneuropathy is similar to the peripheral neuropathy associated with stavudine, zalcitabine, and, to a lesser extent, didanosine. Results of one recent study (Brew et al, 8th CROI, 2001) indicate that elevated lactate levels may distinguish between the disorders. Subjects with stavudine-associated peripheral neuropathy were identified as such if they had been taking stavudine and if symptoms of neuropathy improved after cessation of stavudine use; subjects with HIV neuropathy were identified as such if they were not taking stavudine (none of the subjects was on zalcitabine or didanosine). Increased lactate levels that resolved with treatment discontinuation were found in 13 of 15 patients with stavudine-associated peripheral neuropathy versus 1 of 6 with HIV neuropathy.

> Many of the effects associated with mitochondrial toxicity are difficult to distinguish from effects associated with HIV infection itself

Nerve biopsy findings have indicated the presence of damaged mitochondria in patients with neuropathy associated with didanosine, stavudine, or zalcitabine. A study using the in vitro axon degeneration model indicates that toxicities of zalcitabine and didanosine, but not stavudine, were correlated with mtDNA depletion.

Pancreatitis

There are a number of factors predisposing to risk of pancreatitis in HIV-infected patients, including some opportunistic infections (eg, cytomegalovirus infection, *Mycobacterium avium* complex infection, and tuberculosis), malignancies, hypertriglyceridemia, high alcohol intake, and use of pentamidine.

Antiretrovirals associated with risk are the nRTIs didanosine and stavudine, with risk increasing when either is used in combination with hydroxyurea. Some cases of pancreatitis in HIV-infected patients receiving nRTIs have been associated with lactic acidosis. Didanosine does not produce pancreatic toxicity in rats. A toxic effect on mitochondria has, however, been observed in vitro in human pancreatic cell culture systems. A relationship of pancreatitis to mitochondrial dysfunction is suggested by characteristics of Pearson's marrow-pancreas syndrome, a condition associated with mtDNA deletions. These include cytopenias, exocrine dysfunction (malabsorption), pancreatic fibrosis, and acinar cell atrophy. Didanosine's toxicity extends to other exocrine glands, eg, the salivary glands, with elevated levels of salivary amylase and the sicca syndrome.

Proximal Renal Tubular Dysfunction

The kidney features a large mitochondrial population that is needed to satisfy the high energy requirement for driving sodium-potassium pumps in the proximal renal tubule. Inherited mitochondrial disease affecting the kidney most commonly takes the form of proximal renal tubular dysfunction (PRTD) or Fanconi's syndrome. The nucleotide reverse transcriptase inhibitor (nt*R*TI) adefovir, which is no longer being investigated for the treatment of HIV disease but is in development for potential use in hepatitis B virus infection, was associated with PRTD in approximately 50% of HIV-infected patients in a large multicenter trial (Kahn et al, JAMA, 1999). A recent case report of a patient with adefovir-associated PRTD described abnormal mitochondria, deficiency of cytochrome C oxidase (a mitochondrial enzyme coded by mtDNA), and reduced mtDNA in a kidney biopsy (Tanji et al, *Hum Pathol*, 2001).

Hepatic Steatosis and Lactic Acidosis

Cases of liver failure with macrovesicular and microvesicular steatosis have been

reported in association with nRTI therapy. It is believed that mitochondrial dysfunction in the liver results in inhibition of fatty acid oxidation, resulting in accumulation of triglycerides and fatty acids in vesicles. Lactic acidosis is a feature of the hepatic steatosis syndrome. Inhibition of oxidative metabolism results in anaerobic metabolism and increased lactate production, and there are data demonstrating that nRTIs increase lactic acid production in cell culture systems (Chen et al, *Mol Pharmacol*, 1991). The incidence of hepatic steatosis is relatively low (1.3-3.9 cases per 1000 patient-years of nRTI use) but the case fatality rate is high (Fortgang et al, *Am J Gastroenterol*, 1995; John et al, AIDS, 2001).

There appears to be a spectrum of lactate abnormalities in HIV-infected patients. A recent study has indicated a frequency of milder symptomatic hyperlactatemia (defined as reproducible hyperlactatemia with abdominal symptoms or increased alanine aminotransferase level or both) of 14.5 per 1000 patient-years on nRTIs (Lonergan et al, 8th CROI, 2001). The frequency in patients receiving stavudine was 26 per 1000 patient-years, compared with 1.9 per 1000 patient-years of exposure to other nRTIs. Of 6 patients with stavudine exposure in this study undergoing liver biopsy, 5 had steatosis, suggesting a possible link between steatosis and hyperlactatemia.

The prevalence of asymptomatic hyperlactatemia in patients on nRTIs has been estimated at 8% to 21% (Vrouenraets et al, XIII Int AIDS Conf, 2000; Harris et al, *Antivir Ther*, 2000); mild lactate increases are common in patients beginning potent antiretroviral therapy including an nRTI (John et al, AIDS, 2001). Mild elevations in lactate levels are also found in a smaller percentage of antiretroviral-naive patients. The spectrum of clinical findings observed in patients with hyperlactatemia (Figure 2) ranges from an asymptomatic presentation associated with lactate levels of 2.1 to 5 mmol/L, to mild to moderate symptoms associated with lactate levels of 5 to 10 mmol/L, to fatal lactic acidosis and steatosis with levels above 10 mmol/L.

Risk of progression to serious complications, which is associated with lactate levels above 10 mmol/L, does not appear to be significant in patients with asymptomatic, mild elevations in lactate (levels of 2.1-5 mmol/L). Risk of progression remains undefined in those with greater elevations (5-10 mmol/L) and mild-moderate symptoms, whereas there is high risk of serious complications in patients with lactate levels above 10 mmol/L.

Recent case reports have prompted some concern over the potential for steatosis and pancreatitis in patients receiving nRTIs and treatment with interferon alfa and the nucleoside analogue ribavirin. In a report on 2 patients with hepatitis C virus (HCV) and HIV coinfection who were receiving interferon alfa/ribavirin (Lafeuillade et al, *Lancet*, 2001), steatosis, pancreatitis, diabetes, weight loss, and increased lactate level were observed in 1 patient receiving stavudine/didanosine/saquinavir, and steatosis, increased amylase level, and increased lactate level were observed in the other patient receiving stavudine/didanosine/lamivudine. Subsequently, pancreatitis was reported in 3 additional patients on didanosine who were also receiving interferon alfa/ribavirin treatment. Of note, there is literature from the 1980s showing that ribavirin reduces levels of intracellular dATP, thus enhancing didanosine activity (and toxicity).

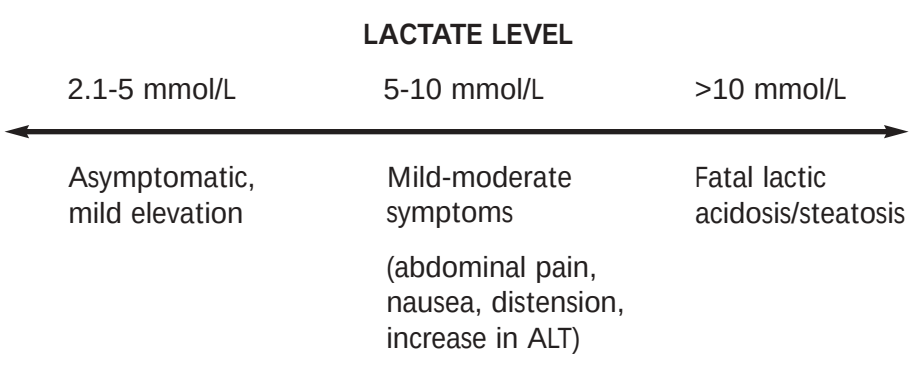

Figure **2.** The spectrum of clinical findings observed in patients with hyperlactatemia. ALT indicates alanine aminotransferase.

Possible Clinical Manifestations of nRTI-Related Mitochondrial Toxicity

Possible, but as yet unproven, clinical manifestations of nRTI-related mitochondrial toxicity include hematologic toxicity, lipodystrophy and lipoatrophy, and osteopenia.

Hematologic Toxicity

There is at least a superficial resemblance of the hematologic abnormalities observed in patients with Pearson's marrow-pancreas syndrome to the anemia and neutropenia associated with zidovudine (Lewis and Dalakis, *Nat Med*, 1995). However, data from in vitro studies of the effect of zidovudine on mtDNA in bone marrow progenitor cells are conflicting. The mechanisms of zidovudine-associated hematologic toxicity remain unclear despite prolonged use of the drug. Mechanisms other than mitochondrial toxicity (eg, effects on heme metabolism) could also be hypothesized.

Lipodystrophy and Lipoatrophy

The phenotype of lipoatrophy in HIV-infected patients receiving antiretroviral therapy at least superficially resembles that in Madelung's disease (multiple symmetric lipomatosis), which is associated with mtDNA mutations. It has been hypothesized that mitochondrial toxicity of adipocytes associated with antiretroviral therapy may lead to adipocyte apoptosis and thus lipoatrophy (Brinkman et al, *Lancet*, 1999; Kakuda et al, AIDS, 1999). Some support for this hypothesis is provided by the following findings from studies in patients receiving therapy: an association of lipoatrophy with hyperlactatemia and liver dysfunction in a patient series (Carr et al,

AIDS, 2000); abnormal adipocyte mitochondria on fat biopsies in a small number of patients with lipoatrophy (Mallal et al, AIDS, 2000); a 60% frequency of decreased mtDNA levels in subcutaneous fat biopsies (neck, abdomen, thigh) in patients with lipodystrophy versus a frequency of 0% to 30% in patients without lipodystrophy or in HIV-seronegative subjects (Shikuma et al, AIDS, 2001); and the association of nRTI use with a decrease in mtDNA (mean, 44%) in fat biopsies in buttocks (Walker et al, *JAIDS*, 2000).

Osteopenia

In one study of 221 HIV-infected men (32 were antiretroviral-naive and 189 were receiving nRTI-containing regimens), osteopenia was associated with asymptomatic hyperlactatemia (odds ratio, 2.39 per 1 mmol/L increase in lactate level) and lower body weight prior to starting antiretroviral therapy (Carr et al, AIDS, 2001). It has been hypothesized that osteopenia may result from mitochondrial dysfunction in bone. Osteopenia has been reported in HCV-infected patients without HIV infection receiving ribavirin/interferon alfa therapy (Solis-Herruzo et al, *J Hepatol*, 2000).

Overview

There are many issues remaining to be clarified about the effects of nRTIs on mitochondria and the potential for clinical manifestations of these effects. Some of these issues involve the differing adverse effects among nRTIs that may be associated with mitochondrial toxicity—eg, why is zidovudine associated with myopathy, whereas stavudine is associated with neuropathy?

Manifestations of mitochondrial defects are likely to occur in tissue dependent on oxidative phosphorylation, and it may be that nRTIs differ in capacity to penetrate different tissues or that the cellular kinases that phosphorylate nRTIs function differently for different drugs and in different tissues. Further, the different nRTIs have been reported to have different magnitudes of inhibitory effect on gamma polymerase in vitro (Martin et al, *Antimicrob Agents Chemother*, 1994), although effects in vivo remain undefined.

Similarly, there may be differences among nRTIs regarding the ability of gamma polymerase to proofread and excise the nRTI once it is incorporated into the DNA chain. It has been reported that lamivudine, for example, is readily recognized and excised after incorporation. It also remains unclear whether nRTIs have additive or synergistic effects on mitochondria when used in combination. Finally, it is not understood why only some patients appear to have mitochondrial toxicity or clinical manifestations of such toxicity. In addition to potential drug effects, roles in this regard may be played by HIV infection itself, HCV coinfection, alcohol use, or genetics.

In summary, inhibition of mtDNA polymerase by nRTIs may explain many of the toxicities associated with drugs in this class. Data from cell culture systems and animal studies and, in some cases, biopsy studies in humans, provide some support for this hypothesis. However, the in vitro data are sometimes conflicting with respect to effects of the nRTIs, and it needs to be emphasized that generalizations from these data to potential in vivo effects in humans may not be appropriate.

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Suggested Reading

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