Defective mitochondrial DNA replication and NRTIs: pathophysiological implications in AIDS cardiomyopathy

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DESPITE THE AVAILABILITY of newer and more effective antiretroviral therapy (20) and some promise from human immunodeficiency virus (HIV) vaccine studies (39), the acquired immunodeficiency syndrome (AIDS) epidemic causes increasing global mortality. At present, nucleoside reverse transcriptase inhibitors (NRTIs) in combination with other antiretrovirals (highly active antiretroviral therapy, HAART) are the cornerstones of AIDS therapy, but their extensive use has brought serious side effects to light, including cardiomyopathy (CM) (134).

Clinical experience, pharmacological, cellular, and molecular biological evidence links altered mitochondrial DNA (mtDNA) replication to the toxicity of NRTI (8, 56, 57, 67, 68, 115, 132) in many tissues. mtDNA replication defects and mtDNA depletion in target tissues are observed clinically and experimentally. A working hypothesis explains the varied clinical side effects and invokes mitochondrial toxicity from NRTIs in HAART. Organ-specific pathological changes or diverse systemic effects result from and are frequently attributed to HAART, in which NRTIs are included (5, 7, 16, 35, 53, 81, 90, 96, 110, 117, 124).

Mitochondrial toxicity from NRTIs was established by clinical, in vitro, and in vivo investigations that related mtDNA depletion in target tissues to antiretroviral treatment. More recently, HAART combinations caused mitochondrial toxicity, including lactic acidosis and mtDNA depletion (16, 23). As the AIDS epidemic continues and as survival with HIV infection is prolonged by treatment with HAART, long-term side effects may be more commonly observed. The risk-to-reward ratio unambiguously favors treatment with HAART because AIDS is fatal in the absence of treatment. This editorial examines some proposed mechanisms of NRTI mitochondrial toxicity with respect to key cell biological, pathological, and pharmacological events and relates those events to the development of CM in AIDS.

The first clinical and experimental findings were presented in the early 1990s (1, 24, 65, 69, 76). The shared features of mtDNA depletion and energy depletion became key observations and related the clinical and in vivo experimental findings to inhibition of mtDNA replication by NRTI triphosphates in vitro (49, 62, 77, 86, 98, 132). Subsequent to those observations, another series of observations suggested that mitochondrial energy deprivation is concomitant with or the result of mitochondrial oxidative stress in AIDS (from HIV, for example) or from NRTI therapy itself. In vivo studies with NRTI treatment of inbred mice (4, 27) support this hypothesis, and data from our group and others employing AIDS transgenic mice revealed that oxidative stress results from transgenic expression of HIV tat in the heart and liver (14, 15, 103).

An important correlate is that mtDNA mutations may result from oxidative mtDNA damage, aberrant mtDNA replication, and altered mtRNA transcription. Together, these interlinked events are the cornerstones of the “mitochondrial dysfunction hypothesis” (67) that we applied in the laboratory in models of AIDS CM (66, 71–73, 76). Additionally, the same principles are applicable to mitochondrial toxicity in other targets (70, 74, 75, 77). The “mitochondrial dysfunction hypothesis” (67) clarifies important pathophysiological events in NRTI toxicity. It is reviewed herein in the context of NRTI mitochondrial metabolism and AIDS CM.

It may be reasonably argued that analysis of mechanisms of NRTI-induced mitochondrial toxicity is analogous to approaches that examine defects in genetic mitochondrial illnesses in which the defective mitochondrial gene product, oxidative stress, and the environment contribute to disease pathogenesis (109). It should be noted that clinical and basic reviews of mitochondrial toxicity of NRTIs have been presented elsewhere in which other aspects of the clinical and biological events are detailed (8, 9, 57, 67, 68, 93).

NRTIs AND RELATIONSHIP TO MITOCHONDRIAL TOXICITY IN TARGET TISSUES IN AIDS

At present there are at least five useful NRTIs in the treatment of HIV infection (17). Zidovudine (AZT, 3′-
azido-2',3'-deoxythymidine), zalcitabine (ddC, 2',3'-dideoxyctydine), didanosine (ddl, 2',3'-dideoxynosine), stavudine (d4T, 2',3'-didehydro-3'-dideoxyinosine), and lamivudine (3TC, 3 thiacytidine; cis-1-[2'-hydroxymethyl-5'-(1,3oxathiolanyl)cytosine] are formidable NRTIs that also serve as tools in vitro and in vivo in biomedical and cell biological models of inhibition of DNA polymerase-γ (DNA pol-γ) and mtDNA replication. Today most agents for HIV infection are given in combined antiretrovirals in HAART (134). From the preclinical data alone, the clinical utility of NRTIs may not be ascertained. Although NRTIs with promising antiretroviral activity in vitro continue to be developed (20), some NRTIs are toxic in vivo or clinically. One such agent, fluororodeoxyadenosine (FDDA, 2'-fluoro-2',3'-dideoxyadenosine), went into clinical trial but was discontinued because it later exhibited severe adverse events, including lactic acidosis (19, 42).

Other NRTIs are used to treat common coinfections seen in patients infected with HIV. Chronic hepatitis B infection is a serious and common coinfection that increases morbidity and mortality (47). Accordingly, treatment of chronic hepatitis B infection would benefit such patients. After in vitro studies documented efficacy, the pyrimidine nucleoside analog flarudilane (1-[2-deoxy-2-fluoro-β-D-arabinofuranosyl]-5-iiodouracil, FIAU) was used in clinical trials with promising early results. Later in the trials, FIAU was found to be extremely toxic to the liver, skeletal and cardiac muscle, the pancreas, and the peripheral nerve in treated patients. Mitochondrial toxicity from FIAU was profound. Lactic acidosis and hepatic failure required heroic clinical interventions and necessitated early termination of the trials. Deaths occurred. Abandonment of these compounds as pharmacological agents was subsequently confirmed by findings of mitochondrial toxicity in animal models (70, 118) and in humans (87).

**PREDISPOSITION TO NRTI TOXICITY**

Presently, genetic predispositions to NRTI toxicity or associated somatic mutations that may have pharmacogenetic implications are incompletely understood. Clinical correlates for NRTI toxicity were made in some of the early studies in which AZT liver toxicity was associated with obesity and female gender (22, 96), but more refined correlates were lacking. NRTI toxicity presents a variable and complex diagnostic phenotype in the treated population and mimics key features of mitochondrial diseases. As such, NRTI toxicity may serve as an important model system for relevant pharmacogenomic studies.

For patients with lactic acidemia and treatment with NRTI-containing regimens, a phenotype of mtDNA depletion was found in blood cells (23). Depending on the biological system, deleterious effects on mitochondrial structure and function in selected targets have been documented (68). The specificity of blood cell mtDNA as a surrogate markers for NRTI toxicity (mtDNA depletion) was impeded by the selection of control groups (105). The impact on mitochondrial function in the surrogate tissue remains unclear (11), despite the logic of the working hypothesis. Standard methods for diagnosis suggest examination of plasma lactate or lactate-to-pyruvate ratios (12, 105), but these also require careful sample preparation and handling. Overall, depletion of mtDNA appears to be an important marker of the toxic process and may serve as a diagnostic hallmark (1, 69), as a way to monitor successful HAART therapy (23), and a therapeutic window that enables changes in HAART regimens. The ideal surrogate tissue to monitor mtDNA depletion from NRTIs remains to be determined, but blood samples or other samples may serve as promising, noninvasive tissue sources.

The “DNA pol-γ hypothesis” (68) integrates clinical observations and biochemical, pharmacological, and pathological data into a rational pathophysiological framework. First, the intracellular and intramitochondrial abundance of the NRTI must be sufficient to impact on the intramitochondrial pool of nucleotides. The NRTI triphosphates (when synthesized by cellular enzymes) compete for incorporation into nascent mtDNA with their natural counterparts.

Mitotically quiescent tissues like the myocardium and liver possess intramitochondrial nucleoside kinases for salvage [including thymidine kinase 2 (TK2), the mammalian mitochondrial isoenzyme] (2). These kinases must phosphorylate NRTI sufficiently to provide substrate for downstream phosphorylation to the pharmacologically active (and toxic) moiety. The NRTI triphosphates must be an effective inhibitor of DNA pol-γ so that so that the inhibitory constant (K_i) with DNA pol-γ authentically reflects both enzymological inhibition and tissue toxicity. Inhibition is dependent (in part) on the ability of NRTI triphosphate to compete with the native nucleotide at the nucleotide binding site of DNA pol-γ. Subsequently, its ability to adulterate the nascent mtDNA depends on the incorporation of monophosphate and chain termination of mtDNA. Target tissues must be significantly affected by energy deprivation in the face of depleted mtDNA. This follows the oxidative phosphorylation (OXPHOS) paradigm (125–127) as the phenotype from the toxic events relates to a “dose effect” of mtDNA replication.

Aspects of the DNA pol-γ hypothesis are found in the principles of mitochondrial medicine (83). If the intramitochondrial pool of nucleosides is disrupted, altered energetics may occur. This is seen in the neurogastric syndrome [mitochondrial neurogastrointestinal encephalomyopathy; (95)]. Inefficient monophosphorylation of thymidine in mitochondria (by mitochondrial TK2) results in genetic illnesses with lactic acidosis and muscle weakness (107). Arnold Katz (58) explained the role of mitochondrial alterations in the development of low output congestive heart failure using such reasoning. The inability of mitochondria to function normally in that latter setting related to decreased cardiac performance. It is generally considered axiomatic that many genetic mitochondrial illnesses manifest with a threshold based at least in part to the heteroplasmic effects of the associated mtDNA muta-
tion as stated in the OXPHOS paradigm (125–127). Energy deprivation, possibly the initiating step of NRTI toxicity based on mtDNA depletion, relates decreased energy abundance in tissues (e.g., heart) to decreased functional mitochondria. To that extent, the threshold phenomenon is intimately involved with phenotypic change.

**Nucleotide Pools, NRTIs, and mtDNA Replication**

One important aspect of NRTI toxicity that is part of the DNA pol-γ hypothesis (68) is the requirement of sufficient intermitochondrial NRTI mass to alter mtDNA replication through DNA pol-γ inhibition by NRTI triphosphate. Because the active pharmacological (and toxic) element of AZT is the triphosphate (i.e., AZTTP), and AZTTP is inhibits both HIV reverse transcriptase (34, 88) and mammalian DNA pol-γ in vitro (77), it follows logically that sufficient NRTI triphosphate must be available for inhibition of mtDNA synthesis, depletion of mtDNA (112), and development of toxic manifestations.

For the pyrimidine AZT, phosphorylation occurs in three intracellular steps. TK phosphorylates AZT to AZTMP (detailed below). TK phosphorylates AZT to AZTDP. Nucleoside diphosphate kinase yields the active AZTTP (21) from AZTDP. Accordingly, a key step in the pathophysiology of NRTI pharmacology and toxicity are regulation of natural dNTP and NRTI triphosphate pool sizes that impact on mtDNA replication as well as the maintenance of function of key elements involved in NRTI phosphorylation.

Mitochondrial ribonucleotide reductase is not reported, so import of dNTPs or deoxyribonucleosides (or the analogous NRTIs) into mitochondria must occur. dNTPs are synthesized by the cytosolic ribonucleotide reductase and can be imported directly through the mitochondrial membrane (6). Deoxyribonucleosides are derived from dNTP catabolism and from extracellular sources (104). Import into mitochondria allows for phosphorylation by specific kinases (52).

**Nucleoside Kinases for Nucleoside Processing: Mammalian TK Isoforms and Deoxyguanosine Kinase**

The salvage pathway activates deoxyribonucleosides by sequential phosphorylation to their biological active triphosphates. The first step is usually rate limiting (2). Deoxyribonucleosides are phosphorylated to their respective monophosphates by deoxynucleoside kinases (2). In the cytoplasmic compartment, this is accomplished by deoxycytidine kinase (44) and the isoform TK1, an S phase-regulated kinase expressed in dividing cells (2).

As mentioned previously, in the mitochondrial compartment, TK2 is the isoform that catalyzes the first step in the mitochondrial salvage pathway for pyrimidines, including deoxythymidine, deoxyuridine, and deoxycytidine, and NRTIs like FIAU and AZT. With respect to purines, deoxyguanosine kinase (dGK) initiates salvage in mitochondria for mtDNA synthesis. This 60-kDa mitochondrial enzyme monophosphorylates deoxyguanosine, deoxyadenosine, and deoxyinosine (38, 97).

Whereas TK1 (cytosolic isoform) has relatively low activity in extracts of striated skeletal muscle (29, 30), TK2 (mitochondrial) has higher activity in muscle. Although TK2 phosphorylates AZT, TK2 performs the phosphorylation relatively poorly compared with TK1 (94). TK2 has a broader substrate range and phosphorylates deoxycytidine and 5'-substituted deoxothymidine and deoxycytidine analogs. When compared with TK2, TK1 tolerates more sugar modifications. This allows for phosphorylation of 2',3'-ddN analogs that are less effective substrates for TK2 (94). ddNTPs function as either competitive inhibitors of the natural substrates of polymerases (reviewed in Ref. 89); or lead to chain termination (88, 121). Bovine striated muscle TK2 has been purified to homogeneity (50).

Unlike TK2 (which resides in the mitochondrial matrix) (61), the submitochondrial location of dGK was unknown for some time. A potential mitochondrial targeting peptide (128) suggested that dGK is a bona fide mitochondrial matrix protein. Although the targeting peptide does not guarantee a matrix localization, Erikkson’s group (100) localized dGK to the mitochondrial matrix. Human dGK efficiently phosphorylates dG and dA, whereas TK2 phosphorylates deoxothymidine, deoxycytidine, deoxyuridine. Genetic mutations in dGK result in a mitochondrial phenotype (discussed below).

**NRTI Inhibition of mtDNA Replication and DNA Pol-γ**

Nuclear DNA encodes 80% of the OXPHOS genes (the principal source of myocardial energy). Thirteen OXPHOS gene products are encoded by mtDNA (reviewed in Ref. 125). In contrast to mitochondrial genetic diseases where mutations are documented (126), acquired defects in mtDNA replication resulting from inhibition of NRTI of mtDNA replication may yield phenotypic OXPHOS defects that mimic the genetic illnesses.

It should be understood that mtDNA replication is governed by nuclear encoded polypeptides. DNA pol-γ (the mitochondrial DNA polymerase) is the nuclear-encoded mtDNA replication enzyme in eukaryotic cells. DNA pol-γ extracted from the fly, the frog, and the human reveals significant sequence homology. DNA pol-γ contains two subunits. One subunit (25–140 kDa) contains polymerase and exonuclease catalytic activity. An accessory subunit of 41–55 kDa is required for processive synthesis (10, 40, 48, 78, 129, 131). Polymerase function in DNA pol-γ is pathophysiologically linked to the “mitochondrial dysfunction hypothesis” (67). Decreased energy production is secondary to decreased mtDNA abundance and results in a phenotypic changes. When DNA pol-γ kinetics are inhibited by NRTI triphosphates, mtDNA synthesis is inhibited and mtDNA depletion results. Moreover, NRTI toxicity
appear to be cumulative, reinforcing the similarity to mitochondrial genetic diseases.

DNA pol-γ is processive because of its accessory subunit. High processivity allows the enzyme: template complex to replicate the mitochondrial genome completely in one binding event (78). Heteroplasm is an intracellular or intramitochondrial mix of normal and mutant mitochondrial DNA molecules that ultimately may reflect a phenotype (40, 129). With high DNA pol-γ processivity, deletion mutants (intrinsically smaller, truncated mtDNA templates) may be replicated more quickly and efficiently than the native mtDNA counterparts (78, 129).

GENETIC mtDNA DEPLETION SYNDROMES
MOLECULAR AND PHENOTYPIC SIMILARITIES TO
NRTI MITOCHONDRIAL TOXICITY

Analogies may be drawn between genetic mtDNA depletion syndromes (MDS; OMIM 251880) and mtDNA depletion caused by NRTI therapy in AIDS. It should be emphasized that in the genetic MDS, quantitative mtDNA depletion is the critical point not necessarily the accumulation of mtDNA mutations. Like NRTI-induced mitochondrial toxicity, MDS are heterogeneous, autosomal-recessive disorders with tissue-specific reduction in mtDNA abundance (46, 85, 92, 123). Clinical manifestations in the hepato cerebral form of MDS include progressive liver failure, neurological abnormalities, hypoglycemia, and increased plasma lactate. Target tissues show decreased activity of respiratory chain complexes (I, III, IV, and V) and mtDNA depletion (116). This genetic syndrome shares features with NRTI toxicity. Treatment with AZT depletes mtDNA in the skeletal muscle of humans and rodents (1, 69), and FIAU treatment causes similar events in woodchucks and humans (87, 115).

TK2 mutations represent an etiology for mtDNA depletion and have been associated syndromically with that finding. Two substitution mutations in TK2 (His488Asn; Ile181Asn), resulted in a phenotype of infantile myopathy and mtDNA depletion in muscle (107). TK2 activity in muscle mitochondria was reduced to 14–45% of that found in healthy controls. This emphasizes the importance of the mitochondrial dNTP pool in the pathogenesis of mtDNA depletion and suggests a relationship to the myopathy found with mtDNA depletion caused by AZT administration (24).

A single-nucleotide deletion (204delA) was identified within the coding region of dGK that segregated with the disease in three kindreds (84). mtDNA depletion and mutated dGK suggests that the salvage-pathway enzymes are involved in the maintenance of balanced mitochondrial dNTP pools. Muscle weakness, liver failure, and multisystem involvement with lactic acidosis all are described. Many of these findings resemble those of NRTI mitochondrial toxicity (67, 68) where heart muscle, skeletal muscle, liver, and peripheral nerve have been identified as targets.

Other genes that control mtDNA replication may play important roles pathogenetically in heritable diseases of mtDNA depletion. Among the gene targets included are adenine nucleotide translocator [ANT1; locus 4q34–35; (59)], thymidine phosphorylase [locus 22q13.32qter; (95)], an unidentified gene [at 3p14–21; (60)], and DNA pol-γ (15q22–26; (106)].

DNA sequences obtained from patients with progressive external ophthalmoplegia (PEO) revealed a heterozygous A → G mutation at codon 955 (Y955C), a highly conserved residue at the DNA pol-γ active site (122). A recent series of experiments from Copeland’s group (102) at the National Institute of Environmental Health Sciences indicated that error-prone DNA polymerase with Y955C mutation is associated with decreased stringency for dNTPs and relates to PEO. Pre-disposition to accumulation of mtDNA mutations (as in PEO with Y955C) follows a course of progressive, cumulative effects. Identification of exonuclease-deficient DNA pol-γ (82), the Y955C DNA pol-γ mutation (102), and a transgenic mouse described by Zassenhaus and colleagues (135), in which exonuclease-deficient DNA pol-γ overexpressed in the heart was associated with CM, mtDNA point mutations, deletions, and direct repeats points to the phenotypic correlate of the genetic alterations.

Mutations that reduce fidelity of DNA pol-γ cause mtDNA diseases through ineffective or mutagenic mtDNA replication. (122). “Twinkle” is another gene encoding a putative mitochondrial helicase that is causally related to PEO with mtDNA deletions (113). Alternatively, ANT1 and phosphorylase mutations may result in a similar mtDNA depletion phenotype based on alterations of dNTP pools (59, 95). Imbalance in mitochondrial nucleotide pools enhances base substitution errors in DNA pol-γ in vitro (64, 130). In other studies, Wallace’s group (31) found mtDNA rearrangements and increased reactive oxygen species in ANT−/− knockout mice. These suggested that oxidative damage was integral to mtDNA defects (31).

On a biochemical basis, one potential defense against NRTI toxicity that is intrinsic to DNA pol-γ function is in the 3′ → 5′ exonuclease activity of the enzyme. This exonucleolytic function (63, 64) is inhibited by nucleoside 5′-monophosphates (55).

OXIDATIVE STRESS AND ITS RELATIONSHIP TO MtDNA ALTERATIONS AND HIV INFECTION

Although energy depletion from altered mtDNA replication in NRTI toxicity is a logical consequence (68–70, 72, 74–77), related events of oxidative stress also impact on energetics in striated muscle (27, 133) and mtDNA replication, on heart failure in general, and on HIV infection and AIDS. In the context of NRTI toxicity, oxidative stress is an imbalance between the production of reactive oxygen species (such as superoxide, hydrogen peroxide, lipid peroxides, hydroxyl radical, and peroxynitrite) and the antioxidant defenses that prevent damage to cells (3). Mitochondria serve as both a logical target for the stress and as a source of the biochemical moieties that contribute to or cause it. The proximity of mtDNA, mtRNA, mitochondrially and nu-
clear-encoded proteins, and lipids to the highest gradient of oxidants (near the source) is a crucial factor as well. Thus it is reasonable to implicate mitochondria and oxidative stress in some aspects of the toxicity of NRTIs. Conversely, it may be reasonable to use therapeutic strategies that are focused on the prevention of oxidative stress as a means to prevent, attenuate, or ameliorate NRTI toxicity. Some studies in our laboratories focus on this important issue. The role of oxidative stress in the development of NRTI toxicity has been reviewed in greater detail (67).

CM AND NRTIs IN AIDS

NRTIs cardiovascular toxicity, particularly from AZT, is a bona fide complication of therapy. A cumulative mitochondrial skeletal myopathy occurred in AZT-treated, adult AIDS patients (24, 41, 120). “Ragged red fibers” (111) and ultrastructural paracrystalline inclusions (24) were observed in muscle samples and indicated subsarcolemmal accumulation of mitochondria in the skeletal muscle with long-term, high-dose AZT treatment. Mitochondria were enlarged and swollen ultrastructurally and contained disrupted cristae and occasional paracrystalline inclusions (65, 68, 76, 101). Extracts of muscle biopsy specimens of AZT-treated patients revealed decreased skeletal muscle mtDNA. Mitochondrial dysfunction in AZT-induced myopathy, results in inefficient utilization of long-chain fatty acids for Β-oxidation. Fat droplets accumulated. AZT myopathy developed at least 6 mo of therapy and occurred in up to 17% of treated patients (25, 99). Dalakas and colleagues (24, 25) showed that it occurs with the high-dose therapy and with current low-dose regimens.

The case for CM resulting from NRTIs in AIDS is less clear. CM related to AZT and/or other antiretroviral therapy has been reported. Interestingly, discontinuation of NRTIs resulted in improved left ventricular function (45), perhaps the earliest report of planned therapeutic interruption of antiretroviral therapy. Clinical features of AZT CM resemble some of those described for CM of other etiologies but with the addition of AIDS or HIV infection. Data suggest that cardiomyopathy in the setting of AIDS has an ominous prognosis (32), but the direct relationship to NRTI or HAART therapy has not been made. Clinical features of CM from NRTIs include congestive heart failure, left ventricle dilatation, and reduced ejection fraction. Endomyocardial biopsy data in AZT CM is incomplete. One small study showed ultrastructural changes of intramyocytic vacuoles, myofibrillar loss, dilated sarcoplasmic reticulum, and disruption of mitochondrial cristae (26), features consistent with mitochondrial CM.

The role of NRTI in the development of CM in HIV-infected children also remains controversial. Clinical studies are inconclusive. In studies of pediatric patients with AIDS and of neonates treated with AZT, both in utero and perinatally, Lipshultz and colleagues (79, 80) reported that impaired cardiac function was not attributed to AZT. Again, myocardial biopsy findings could suggest or disprove an etiology but were not included. Additionally, because myopathy is uncommon in AZT-treated children with AIDS (51), it may be reasonable to expect that the pediatric striated and cardiac muscle tissue responds differently to NRTI-related toxicity. Other reports (28, 79, 80) suggest that AZT CM in pediatric patients may be more prevalent than previously recognized. In contrast to some of the above studies, a causal relationship between NRTI therapy and cardiac dysfunction was suspected. Interesting correlative data come from in vivo studies in primates using pregnant Erythrocebus patas. Neonatal E. patas treated with AZT in utero reveal features of mitochondrial toxicity to heart and skeletal muscle (36, 37) that resemble those described in experimental systems with rodents. Although not extensively evaluated, the effects of NRTI-induced oxidative stress in arteries may impact on the development of CM as well.

NRTI TOXICITY AND OTHER TISSUE TARGETS

On the basis of the OXPHOS paradigm (126) and our working hypothesis (67, 68), it is logical to expect mitochondrial events from NRTIs to impact on diverse tissues. Hepatic toxicity from AZT, ddI, and ddC was reported (13, 33, 54). It is presumed to relate to toxicity to liver mitochondria. Fatal hepatomegaly with severe steatosis (33), severe lactic acidosis (13), and adult Reye’s syndrome (54) in AZT-treated HIV seropositive patients were all pathogenetically linked to AZT-induced hepatotoxicity. Clinical features resembled some of those seen in FIAU toxicity. The prevalence of metabolic abnormalities is increasing in AIDS patients treated NRTI analogs, and the relationships to a variety of metabolic and cardiovascular changes in AIDS are being investigated more closely.

Clinical treatment with certain NRTIs (d4T/3TC) results in anion gap acidosis (91). Moreover, the lactic acidosis/hepatic steatosis syndrome may be more common than previously appreciated in adults (5, 81, 119) and children (16) treated with NRTIs. d4T treatment caused lipodystrophy (108). Mechanisms may involve altered mitochondrial biogenesis and/or oxidative changes and possibly adipocyte apoptosis (43, 68). Recently, we demonstrated arterial dysfunction in FVB/n mice treated with AZT (114), which may be another important target of toxicity. NRTIs have associated peripheral neuropathies as side effects (18, 68). The role of altered mtDNA replication in the development of the clinical manifestations is a major effort in our laboratories.

In summary, NRTI toxicity now is an important clinical problem with long-term significance to AIDS patients. mtDNA replication defects result from NRTI toxicity. Their manifestations clinically are increasing with asymptomatic hyperlactatemia being fairly common in the HIV-infected patient population receiving HAART. Tissue-specific toxicities include skeletal myopathy, cardiomyopathy, peripheral neuropathy, and other changes. Toxicities may be severe enough to limit...
antiretroviral therapy in some cases, but in others, the clinical impact of NRTI toxicity is less clear.

Understanding subcellular mechanisms of handling nucleosides in mitochondria is an important step to elucidate the pathophysiological mechanisms of mitochondrial toxicity from NRTIs. mtDNA and energy depletion, oxidative stress, and mtDNA mutations (articulated in the mitochondrial dysfunction hypothesis) appear crucial, but the initiating event remains to be clarified. Future studies will pinpoint subcellular mechanisms of NRTI toxicity, susceptible patient populations in which NRTI toxicity may be prevalent, genetic predispositions for development of NRTI toxicity, and pharmacological approaches to prevent or diminish this important side effect.

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