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Review

Mitochondrial dysfunction and nucleoside reverse transcriptase inhibitor therapy: experimental clarifications and persistent clinical questions

William Lewis*

Department of Pathology, Emory University, Room 7117, 1639 Pierce Drive, Atlanta, GA 30030, USA

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Abstract

Nucleoside reverse transcriptase inhibitors (NRTIs) in combination with other antiretrovirals (HAART) are critical in current AIDS therapy, but mitochondrial side effects have come to light with the increased use of these compounds. Clinical experience, pharmacological, cell and molecular biological evidence links altered mitochondrial (mt-) DNA replication to the toxicity of NRTIs in many tissues, and conversely, mtDNA replication defects and mtDNA depletion in specific target tissues are observed. 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*. Subsequent to those findings, other 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. With increased use of NRTIs, *mtDNA mutations* may become increasingly important pathophysiologically. One important future goal is to prevent or attenuate the side effects so that improved efficacy is achieved.

Keywords: Mitochondrial dysfunction; HIV; Nucleoside reverse transcriptase toxicity; Treatment of NRTI mitochondrial toxicity

1. Introduction

Basic and clinically oriented reviews exist in the literature that focus attention on mitochondrial (mt) DNA replication and related events in nucleoside reverse transcriptase inhibitors (NRTI) toxicity in AIDS therapy (Brinkman, 2001; Brinkman et al., 1998, 1999, 2000; Casademont et al., 2002; Honkoop et al., 1997; Johnson et al., 2001; Kakuda, 2000; Kakuda et al., 1999; Lewis, 2003a,b; Lewis, 2000; Lewis et al., 2001; Lewis and Dalakas, 1995; Moyle, 2000; Walker, 2001; Wright and Brown, 1990). Despite intense interest and significant knowledge that relates some of pathophysiological mechanisms of mitochondrial toxicity from NRTIs, a number of questions remain to be answered clinically and experimentally. These questions include:

- What can we predict clinically from in vitro studies about NRTI mitochondrial toxicity?
- What is the clinical impact of mtDNA depletion in AIDS?
- Is there a way to treat or prevent or treat NRTI mitochondrial toxicity?
- Does NRTI toxicity have tissue specificity?

This brief review highlights clinical and basic evidence for NRTI mitochondrial toxicity. Furthermore, it relates known pathophysiological events to the clinical events.

2. mtDNA replication and DNA polymerase-γ

Nuclear DNA encodes the majority of the oxidative phosphorylation genes (OXPHOS; the principal source of myocardial energy). However, 13 OXPHOS genes are encoded by mtDNA (reviewed in (Wallace, 1992a)). Defects in expression or function of mitochondrially encoded polypeptides become the foundation for the field of mitochondrial *genetic* diseases (Luft, 1994), and point a way to examine complex diseases (Wallace, 1999). *Acquired* defects in mtDNA replication occur as secondary effects of defective mtDNA replication (Ponamarev et al., 2002; Zeviani, 2001), and may be modeled pharmacologically by inhibition of mtDNA replication. Accordingly, certain events in NRTI

* Tel.: +1-404-712-9005.
E-mail address: wlewis@emory.edu (W. Lewis).

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toxicity may yield phenotypic OXPHOS defects that mimic genetic mitochondrial illnesses, and conversely, the toxicity of NRTIs may provide model systems to study altered mtDNA replication. Highlights of some points of mtDNA replication at the level of DNA polymerase is presented below as an introduction to mitochondrial DNA replication and its dysfunction.

DNA polymerase-γ (DNA pol-γ) is the eukaryotic mtDNA replication enzyme (Meyer and Simpson, 1968; Bertazzoni et al., 1977). DNA polymerase-γ is encoded by the nuclear genome and it contains two subunits (Wernette and Kaguni, 1986; Insdorf and Bogenhagen, 1989; Gray and Wong, 1992; Wang et al., 1997; Carrodeguas et al., 1999; Lim et al., 1999). DNA pol-γ extracted from fly, frog and human are highly homologous. The accessory subunit provides tighter DNA binding of the complex thus allowing highly *processive* DNA synthesis (Lim et al., 1999).

Polymerase function is fundamental to NRTI toxicity in the "mitochondrial dysfunction hypothesis" (Lewis et al., 2001) since decreased energy production follows decreased mtDNA abundance. When DNA pol-γ activity is inhibited by NRTI triphosphates, mtDNA depletion results. It is reasonable to conclude that NRTI toxicity may be cumulative and toxic manifestations increase with duration of exposure in ways that are analogous to those seen with genetic diseases of mtDNA replication.

Deletion mutants (truncated mtDNA templates) may be replicated more quickly and efficiently than native mtDNA counterparts (Wang et al., 1997; Lim et al., 1999) owing to enzyme processivity. Abundance of defective mtDNA may reach a threshold of energy depletion like that of heritable mitochondrial illnesses, including those that include mtDNA depletion (Wallace, 1992a; Moraes et al., 1991; Wallace, 1992b).

3. Mitochondrial dysfunction, DNA pol-γ hypothesis and NRTIs

The "mitochondrial dysfunction hypothesis" (Lewis et al., 2001) is an expansion of our earlier work (Lewis and Dalakas, 1995) articulated as the "DNA pol-γ hypothesis" and the work of other investigators (Wright and Brown, 1990; Parker and Cheng, 1994). The "DNA pol-γ hypothesis" (Lewis and Dalakas, 1995), oxidative stress, and *acquired* mtDNA mutations are incorporated into a pathophysiological continuum related to energy depletion. *Acquired* mitochondrial diseases from NRTI toxicity may affect mtDNA replication at the level of competition with native nucleotide pools and nucleotide binding site of the polymerase.

They ultimately result in depletion of mtDNA in affected tissues. The phenotypic results included deleterious effects on mitochondrial structure and function in selected targets, much like what may be expected in some genetic mitochondrial illnesses. Depletion of mtDNA was important to the toxic process mechanistically and appeared to be a diagnostic hallmark (Arnaudo et al., 1991; Lewis et al., 1992).

An analogous hypothesis (Katz, 1998) explained the role of mitochondrial defects in the pathophysiology of congestive heart failure and decreased cardiac performance based on the principle of "energy starvation." Energy deprivation, possibly the initiating step of NRTI toxicity based on mtDNA depletion, relates decreased energy abundance in tissues (e.g. heart) to decreased abundance of normal, functional mitochondria. Wallace's *OXPHOS paradigm* (Wallace, 1992a,b) indicates that tissue requirements for oxidative phosphorylation and threshold effects of dysfunction appear to be integral to the development of symptoms in genetic illnesses of mtDNA, and similar events may occur in acquired defects of mtDNA that relate to NRTIs.

4. Oxidative stress as a pivotal event in NRTI toxicity

Energy depletion from altered mtDNA replication in NRTI toxicity is a logical consequence (Lewis and Dalakas, 1995; Lewis et al., 1991, 1994a,b, 1992, 1996, 1997, 2000, 2001). However, the effects of *oxidative stress* may amplify some of the pathophysiological and phenotypic events in NRTI toxicity to mitochondria. Oxidative stress (operationally defined here as an imbalance between the production of reactive oxygen species i.e. superoxide, hydrogen peroxide, lipid peroxides, hydroxyl radical and peroxynitrite) requires effective cellular antioxidant defenses that prevent damage from those moieties (Betteridge, 2000). Mitochondria are targets for oxidative stress based on their ability to generate reactive oxygen species and may be primarily involved in oxidative stress associated with AIDS treatment. Chronic AZT treatment induces oxidative damage of skeletal muscle in mice (de la Asuncion et al., 1998) and in rats treated acutely with AZT (Szabados et al., 1999).

Aspects of the importance of oxidative stress have been extensively reviewed recently (Droge, 2002) and we addressed the role of NRTI toxicity in oxidative stress (Lewis et al., 2001). It should be understood that the impact of HIV infection per se on the development of oxidative stress in the condition of HIV infection and NRTI treatment has been known for some time but has been relatively under-explored despite documentation of oxidative stress as a feature of HIV infection in which protein catabolism (Droge et al., 1994a), particularly affecting cysteine may impact on general well being as well as specific immune functions of lymphocytes (Droge et al., 1994a,b,c). It is possible to conclude that oxidative stress may serve as a central pathophysiological pathway for cellular damage in AIDS and its treatment.

5. Mutations of mtDNA

Hepatic mtDNA from rat exhibits two orders of magnitude greater oxidative DNA damage than does hepatic

nuclear DNA. Differences in oxidative damage between nuclear DNA and mtDNA may relate to (1) lack of known repair enzymes for mtDNA error excision; (2) a lack of histones protecting mtDNA; and (3) a subcellular proximity of mtDNA to these oxidants. Exposure of DNA to superoxide-generating systems causes extensive strand breakage and degradation of deoxyribose (Brawn and Fridovich, 1981). Peroxynitrite is a potent initiator of DNA strand breaks (Szabo et al., 1996) that causes DNA base modifications (Spencer et al., 1996). On a mass action basis, random mtDNA mutations would likely inactivate complex I first, due to the significant contribution from mtDNA-encoded elements to the makeup of the complex. Furthermore, deficiency of complex I proteins could amplify superoxide formation and increase oxidative stress (Cortopassi et al., 1996).

Oxidation of mtDNA by hydroxyl radicals results in the formation of the oxidized base 8-hydroxydeoxyguanosine (8-OHdG); 8-OHdG is present in hepatic mtDNA at 16-fold higher levels than corresponding nuclear DNA (Richter, 1988; Richter et al., 1988). In human hearts, similar observations were made (Hayakawa et al., 1992). Base modification can lead to mispairing and point mutation. (Pavlov et al., 1994). It follows stochastically that during any given oxidative event, mtDNA will sustain more damage than nuclear DNA (Yakes and Van Houten, 1997; Ames et al., 1993). The number of oxidative hits in rat DNA is estimated at about 100,000 per cell per day. Enzymes for nuclear DNA repair efficiently remove most, but not all, of the adducts in nDNA (Ames et al., 1993). Although most of the components of a mitochondrial base excision repair system have been identified (Pinz and Bogenhagen, 1998), it is unclear how efficiently this repair removes the wide spectrum of adducts that may occur from oxidative damage. Mitochondrial oxidative damage was supported indirectly by the co-existence of malondialdehyde on (or near) the inner mitochondrial membrane (Fleming et al., 1982). Its interaction with mtDNA could lead to cross-linking, deletion errors in transcription, or mtDNA polymerization.

As mentioned, the abundance of 8-OHdG is higher in mtDNA than nuclear DNA in oxidative stress (Kuchino et al., 1987). 8-OHdG in DNA leads to GC → TA transversions unless repaired (Pavlov et al., 1994; Grollman and Moriya, 1993). This may relate to the abundance of mtDNA deletions (Hayakawa et al., 1992; Hattori et al., 1991). An accumulation of mtDNA defects may result in myocytes with oxidative capacity that varies from normal to severely impaired in a so-called myocardial "bioenergy mosaic" in NRTI-treated cells as that seen in the aging heart in absence of NRTI therapy or HIV infection (Linnane et al., 1992). The pathological phenotype may be absent histochemically and a spectrum of activity may be seen in tissues (Muller-Hocker, 1989). Pathophysiological events would not occur until the threshold of damage were severe enough to impact on organ function (Wallace, 1992a).

6. Mitochondrial dysfunction and mtDNA depletion: relationship to nucleoside reverse transcriptase inhibitor (NRTI) pharmacology and biochemistry

Organellar toxicity of mitochondria as an important side effect of NRTI therapy in AIDS is linked pathophysiologically to clinical and experimental settings. Early observations were based in vitro (Chen and Cheng, 1989), animal experiments confirmed those findings in vivo (Lewis et al., 1991, 1992; Lamperth et al., 1991) clinical correlations were found in AIDS patients (Arnaudo et al., 1991; Dalakas et al., 2001). Cornerstones of the pathogenesis of mitochondrial toxicity from NRTIs appears first to include *energy deprivation* secondary to mtDNA depletion which has been consistent if not causative event in the pathophysiological phenotype (Arnaudo et al., 1991; Lewis et al., 1992; Dalakas et al., 2001). However, an unambiguous causation is not confirmed. Concomitant with or resulting from mitochondrial energy deprivation is the second key event: *mitochondrial oxidative stress*. As yet, this latter point has been demonstrated in few studies clinically and experimentally (de la Asuncion et al., 1998, 1999), but acceptance is growing for the involvement of oxidative stress. Last, *mtDNA mutations* may result from oxidative mtDNA damage, aberrant mtDNA replication, and altered mtRNA transcription (Gerschenson and Poirier, 2000).

7. Can NRTI agents be categorized in a toxicologically meaningful way?

NRTIs have been divided into classes of mtDNA replication inhibitors according to the relative importance of DNA chain termination, or internalization of the analog into nascent mtDNA and substitution for the natural base (Kakuda, 2000; Wright and Brown, 1990; Parker and Cheng, 1994). One class inhibits mtDNA replication in ways include monophosphate incorporation into mtDNA. With these agents, *competition* with the native nucleotide and NRTI at the nucleotide binding site of DNA pol-γ appears to be a critical event. This class of compound and its toxicity are exemplified in the clinical and basic studies of fialuridine (FIAU) outlined below.

In this model of *competitive inhibition*, fialuridine (1-[2-deoxy-2-fluoro-β-D-arabinofuranosyl]-5-iodouracil; FIAU) monophosphate incorporates into mtDNA as a crucial event. Among NRTIs that may share this characteristic are FIAU, 1-[2-deoxy-2-fluoro-β-D-arabinofuranosyl]-5-iodocytosine (FIAC), 1-[2-deoxy-2-fluoro-β-D-arabinofuranosyl]-5-methyluracil (FMAU), and 1-[2-deoxy-2-fluoro-β-D-arabinofuranosyl]-5-ethyluracil (FEAU). Each demonstrated virucidal efficacy in disease models (Fourel et al., 1990). In the case of FIAU and its metabolites, many triphosphates inhibit mammalian DNA pol-γ competitively in vitro (Lewis et al., 1994b). With antiviral agents possessing these features, *competition* with the native nucleotide and NRTI

at the nucleotide binding site of DNA pol-γ appears to be a critical event and competitive inhibition kinetics may be predicted.

The second type of NRTI is represented by some dideoxynucleosides such as AZT, ddC and D4T. With such agents, 5′-triphosphates are substrates for mtDNA synthesis by DNA pol-γ. They *compete* with the natural nucleotides (as above) and also *terminate* nascent mtDNA chains because they lack 3′-hydroxyl groups (3′-OH) for continued mtDNA polymerization. mtDNA replication defects from NRTI toxicity may be reversible in some cases. Non-dideoxy-NRTIs have substantial molecular similarities to their natural counterparts. Accordingly, these compounds may have potentially hazardous consequences.

Terminally incorporated AZT monophosphate is not removed by the 3′ → 5′ exonuclease of *S. cerevisiae* DNA pol-γ (Eriksson et al., 1995). Similar results were found using porcine DNA pol-γ against dideoxynucleotide termini (Longley and Mosbaugh, 1991). Human DNA pol-γ removes chain terminators poorly as compared to normal nucleotides. AZT-monophosphate is the most persistent chain terminator to DNA pol-γ exonuclease activity (Lim and Copeland, 2001). It is possible that this could be significant mechanistically in the observed mitochondrial toxicity of DNA by AZT and related NRTIs.

8. What is the clinical impact of NRTI mitochondrial toxicity?

With high dose monotherapy, AZT causes a cummulative mitochondrial skeletal myopathy in adult AIDS patients (Dalakas et al., 1990). It is a bona fide complication, (Groopman, 1990; Till and MacDonell, 1990) with characteristic microscopic “ragged red fibers” (Shoubridge, 1994) and ultrastructural paracrystalline inclusions (Dalakas et al., 1990) that result from subsarcolemmal accumulation of mitochondria in the skeletal muscle with long term, high dose treatment in adult AIDS patients. Mitochondria are enlarged and swollen ultrastructurally and contain disrupted cristae and occasional paracrystalline inclusions (Lewis and Dalakas, 1995; Lewis et al., 1991; Lamperth et al., 1991; Pezeshkpour et al., 1991).

A variety of other phenotypic findings are characteristic. Extracts of muscle biopsy specimens of AZT-treated had depleted skeletal muscle mtDNA. Inefficient utililzation of long-chain fatty acids for β-oxidation occurs and fat droplets accumulate. AZT myopathy develops after at least 6 months of therapy and occurs in up to 17% of treated patients (Dalakas et al., 1994; Peters et al., 1993). Dalakas and coworkers showed that it occurs with the high-dose therapy and with current low-dose regimens. In pediatric populations with AIDS, AZT skeletal myopathy is less frequently observed and may be masked by coexisting encephalopathy (Jay and Dalakas, 1994). Despite this important evidence, the exact nature of toxicity of AZT can only be inferred since the biochemical point (i.e. inhibition of DNA pol-γ by AZT triphosphate in vivo would be difficult to ascertain). Clinical improvement accompanies histologic improvement and reversal of zidovudine-induced mtDNA changes, perhaps the key indication of an etiological link (Arnaudo et al., 1991; Dalakas et al., 1994). Exercise decreased muscle phosphocreatine (by ^{31}P nuclear magnetic resonance) in AZT-treated patients (Sinnwell et al., 1995). Abnormal mitochondrial respiratory function was found. Enzyme histochemical analysis of muscle biopsies showed partial deficiency of cytochrome *c* oxidase activity (Dalakas et al., 1994; Chariot and Gherardi, 1991). A high lactate/pyruvate ratio (consistent with abnormal mitochondrial function) is seen in the blood of patients with AZT myopathy (Chariot et al., 1994). Assessment of muscle metabolism in vivo using magnetic resonance spectroscopy showed marked phosphocreatine depletion with slow recovery only in AZT-treated, HIV-positive patients (Sinnwell et al., 1995).

Controversy exists regarding potential mechanisms. The biologic effects of AZT may not reside in defective mitochondrial biogenesis, but in other effects (Szabados et al., 1999; Modica-Napolitano, 1993). Some studies document a lack of correlation between kinetics of inhibition of DNA polymerase-γ by the NRTI triphosphate (Martin et al., 1994), others (Johnson et al., 2001; Feng et al., 2001) are more in agreement with our earlier biochemical findings (Lewis and Dalakas, 1995; Lewis et al., 1994a,b) that were used to support the above animal models. At present, the role of mitochondrial toxicity from this class of drugs appears to be a more accepted outcome (Swartz, 1995).

With respect to mitochondrial toxicity of NRTIs, cardiomyopathy (CM) related to AZT and/or other antiretroviral therapy has been a principal focus of attention of my laboratory for nearly two decades, but we have explored the toxicity of other antiretroviral compounds as well. First, it should be understood that CM is reported in AIDS, but it remains controversial. Features of AIDS CM are shared with the documented AZT myopathy. In support of the working hypothesis, discontinuation of NRTI therapy resulted in improved left ventricular function (Herskowitz et al., 1992) and clinical features of AZT CM occur after prolonged treatment. In contrast to skeletal muscle data in humans, endomyocardial biopsy data is incomplete. One small study showed ultrastructural changes of intramyocytic vacuoles, myofibrillar loss, dilated sarcoplasmic reticulum, and disruption of mitochondrial cristae (d’Amati et al., 1992).

The experimental literature has documented mitochondrial changes in selected tissues from rats, mice, other rodent species and primates with a variety of NRTI dosing schedules. Even using current human therapeutic doses or NRTI doses that are below those currently used clinically, mitochondrial defects have been demonstrated in some tissues (Lewis et al., 1991, 1992, 1997, 2000, 2001; de la Asuncion et al., 1998; Lamperth et al., 1991; Gerschenson and Poirier, 2000; McCurdy and Kennedy, 1998; Tennant et al., 1998; Gerschenson et al., 2000). It should be noted

however, that some NRTIs, like "L-drugs," lamivudine (3TC) and related newer compounds, have not demonstrated significant mitochondrial toxicity despite fairly extensive clinical data (in the case of 3TC). Biochemical support of the differential toxic mechanisms has been proposed by Anderson and coworkers (Feng et al., 2001).

CM may be an important illness in children with AIDS, but as is the case in adults, the impact of AZT on CM remains a controversial issue. In large scale studies of pediatric patients with AIDS and of neonates treated with AZT both in utero and perinatally, Lipshultz et al. reported that impaired cardiac function was not attributed to AZT (Lipshultz et al., 1992, 2000). Myocardial biopsy findings were absent in any of those reported studies. In parallel, it should be emphasized that AZT-skeletal myopathy is uncommon in children with AIDS (Jay and Dalakas, 1994). Contrasting evidence in other reports suggests that AZT CM in pediatric patients may be more prevalent than previously reported (Domanski et al., 1995). In vivo data from *E. patas* treated with AZT in utero suggest some evidence of a mitochondrial toxicity of AZT to heart and skeletal muscle that resembles those features described in experimental systems with rodents (Gerschenson et al., 2000). More recently, mtDNA depletion was identified in cord blood samples from HIV-infected mothers (Shiramizu et al., 2003) treated with NRTIs, but the clinical impact on NRTI mitochondrial toxicity to the newborn remains to be further elucidated.

Hepatic toxicity from AZT, ddI and ddC was reported early in the epidemic, and recent reports continue to point to the mitochondria as toxic targets (Freiman et al., 1993; Chattha et al., 1993; Jolliet and Widmann, 1990). It is presumed to relate to toxicity to liver mitochondria. Fatal hepatomegaly with severe steatosis (Freiman et al., 1993), severe lactic acidosis (Chattha et al., 1993), and adult Reye's syndrome (Jolliet and Widmann, 1990) in AZT-treated HIV seropositive patients were all pathogenetically linked to AZT-induced hepatotoxicity. Studies in primates (Gerschenson et al., 2001) and biochemical data (Johnson et al., 2001) support the toxicity of stavudine to hepatic parenchyma. 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 relationship to a variety of metabolic and cardiovascular changes in AIDS are being investigated more closely.

Treatment with certain NRTIs (d4T/3TC combinations) results in anion gap acidosis (Moore et al., 2000). Moreover, the lactic acidosis/hepatic steatosis syndrome, may be more common than previously appreciated in adults (Lonergan et al., 2000; Boubaker et al., 2000; Ter Hofstede et al., 2000) and children (Church et al., 2000) treated with NRTIs, and stavudine has been suggested as a culprit in these cases. d4T treatment caused lipodystrophy (Saint-Marc et al., 1999). Mechanisms may involve altered mitochondrial biogenesis and/or oxidative changes, and possibly adipocyte apoptosis (Lewis and Dalakas, 1995; Harrison, 1997). What remains controversial is the association with mild, persistent elevation of plasma lactate. Although extensive experimental data may be lacking to support the hypothesis, it is reasonable to consider the possibility of subclinical mitochondrial dysfunction and resulting anaerobic metabolism may relate to this observation. As was the case early in the epidemic, the cumulative impact of NRTI therapy remains to be determined. However, until other therapy becomes available, it remains imperative to treat HIV infection according to established guidelines (DHHS, 2001).

Mitochondrial toxicity from NRTIs may impact on peripheral nervous system function and as the epidemic continues, these side effects are increasingly important. NRTIs have peripheral neuropathies as side effects (Lewis and Dalakas, 1995; Cohen et al., 1994). Dose-related, painful peripheral neuropathies occurred in the majority of patients treated with ddC in doses of 0.03–0.09 mg/kg per day (Yarchoan et al., 1990; Dubinsky et al., 1989; Merigan et al., 1989; Berger et al., 1993). Peripheral neuropathy with ddI was unexpected, based upon preclinical data (Anderson et al., 1994). It was observed in 3–22% (Lambert et al., 1990; Cooley et al., 1990) of patients after 8 weeks. Peripheral neuropathy occurred in 55% of d4T-treated patients after up to 46 weeks treatment. Clinically, distal dysesthesias, areflexia, distal sensory loss, and mild muscle weakness were common. Axonal involvement was present. Sural nerve biopsies for patients with ddC neuropathy showed axonal degeneration and mitochondria with disrupted cristae. These findings resembled those of experimentally induced neuropathy in ddC fed rabbits (Anderson et al., 1994). Lamivudine has an associated peripheral neuropathy (reviewed in (Dalakas, 2001)).

9. Summary

NRTI toxicity to mitochondria in different tissues now is an important clinical problem with longterm significance to AIDS patients. Mechanisms likely relate to energy depletion, oxidative stress, and mtDNA mutations. Analogous to treatment of other serious infectious agents, combinations of multiple anti-HIV-1 drugs are used to target different viral proteins or points in virus-host life cycle (Lange, 1995; De Clercq, 1997) and may create combined toxicities to mitochondria. As current clinical guidelines recommend combined therapy usually containing NRTI (DHHS, 1999, 2001) such regimens may be important to the development of mitochondrial toxicity in new tissue targets as treatment is prolonged with increased longevity from AIDS. Now more than ever it is crucial to examine mechanisms of mitochondrial toxicity from NRTIs, particularly in the era when AIDS has become a relatively manageable chronic illness.

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