

Mitochondrial toxicity of nucleoside analogue reverse transcriptase inhibitors: a looming obstacle for long-term antiretroviral therapy?

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Nucleoside reverse transcriptase inhibitors suppress HIV replication by blocking reverse transcriptase, an RNA-dependent DNA polymerase. These drugs can also affect cellular and mitochondrial DNA polymerases. Mitochondrial DNA polymerase γ is particularly sensitive to nucleoside reverse transcriptase inhibitors, and the majority of adverse effects caused by nucleoside reverse transcriptase inhibitors are most likely caused by mitochondrial dysfunction. This article reviews the recent clinical implications of nucleoside reverse transcriptase inhibitor-induced mitochondrial toxicity and discusses options for management. *Curr Opin Infect Dis* 13:5–11.

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Abbreviations

ABC	abacavir
ddC	zalcitabine
ddI	didanosine
d4T	stavudine
HAART	highly active antiretroviral therapy
HCV	hepatitis C virus
mtDNA	mitochondrial DNA
NRTI	nucleoside reverse transcriptase inhibitors
PI	protease inhibitors
3TC	lamivudine
ZDV	zidovudine

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Introduction

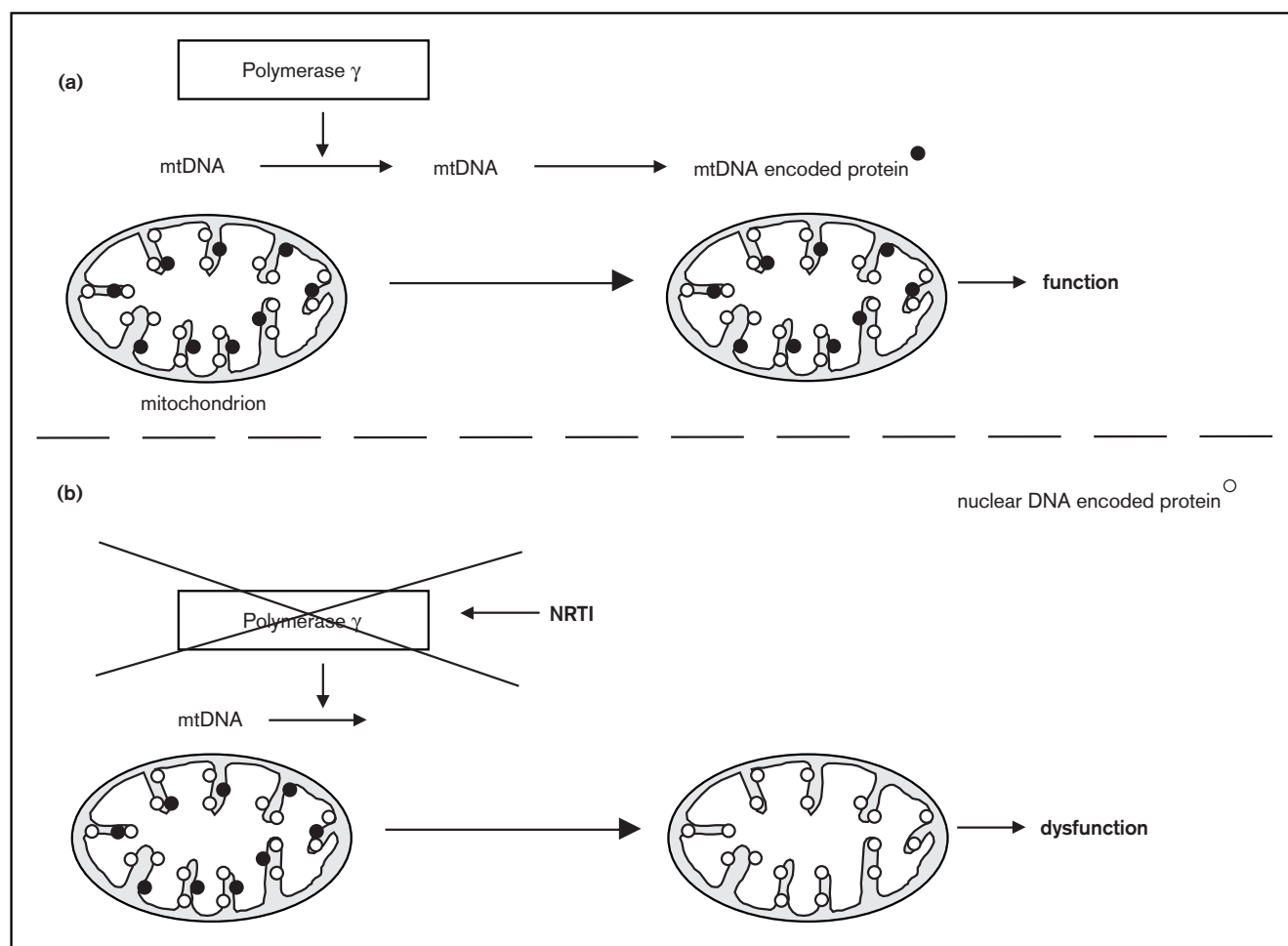
Highly active antiretroviral therapy (HAART) is unlikely to eradicate HIV. Faced with this reality, antiviral therapy may be required for several years or for life until other therapeutic options become available. Consequently, the adverse effects of HAART can become obstacles to continued treatment unless managed appropriately. Central to the HAART regimen is the use of two or more nucleoside reverse transcriptase inhibitors (NRTI). Strong evidence suggests that NRTI cause mitochondrial dysfunction and that this phenomenon may explain the majority of adverse effects associated with these drugs [1,2••]. If long-term treatment with HAART is to be considered, a better understanding of mitochondrial toxicity and its management is needed.

Mechanism of toxicity

NRTI require intracellular phosphorylation to the corresponding triphosphate moiety in order to become active. Phosphorylation is catalysed by either cytosolic or mitochondrial deoxyribonucleotide kinases depending on the cell type. The triphosphorylated NRTI then inhibit HIV reverse transcriptase in two ways: (1) by competing against endogenous deoxyribonucleotides for incorporation; and (2) by prematurely terminating transcription once incorporated. NRTI can also be substrates for two intrinsic DNA polymerases of the host, namely DNA polymerase β and γ . This latter enzyme, DNA polymerase γ , is the sole enzyme responsible for the replication of mitochondrial DNA (mtDNA); the inhibition of mtDNA polymerase γ by NRTI can lead to the depletion or mutation of mtDNA and mtDNA encoded enzymes (Fig. 1). A deficiency of these enzymes will lead to impaired oxidative phosphorylation, with subsequent mitochondrial dysfunction. Tissue damage results not only from impaired energy (decreased ATP) production, but also from the emergence of reactive oxygen radicals (that are normally neutralized by functional mitochondria) and from a disturbed NADH/NAD⁺ equilibrium between the mitochondrial and cellular membrane. Whether all of these mechanisms play an equivalent role or one mechanism is more important than the other is still a matter of debate.

At present, six NRTI are used in clinical practice: zidovudine (ZDV), zalcitabine (ddC), didanosine (ddI), lamivudine (3TC), stavudine (d4T) and abacavir (ABC).

Figure 1. Influence of NRTIs on replication of mtDNA, distributing the mitochondrial function.



(a) Schematic representation of normal replication of mitochondrial DNA (mtDNA) induced by the enzyme DNA polymerase γ , resulting in the production of mtDNA encoded proteins and normally functioning mitochondria. (b) When nucleoside reverse transcriptase inhibitors (NRTI) inhibit DNA polymerase γ , the production of mtDNA and mtDNA encoded proteins will be interrupted, finally leading to dysfunctioning mitochondria. Reproduced with permission [3**].

In-vitro inhibitory constants for DNA polymerase γ have been determined but differences in assays make a comparison of the inhibitory constants difficult [2**]. Martin *et al.* compared the inhibitory constants for several NRTI triphosphates, including the six available NRTI, using a single assay [4]. On the basis of these data, a hierarchy of the NRTI in their potency to induce mitochondrial dysfunction *in vitro* can be made (ddC > ddI > d4T >> 3TC > AZT > ABC). The difficulty in extrapolating these data to clinical toxicity is the fact that the efficiency in phosphorylation among NRTI also differs. For example, the active form of ddI (ddA-TP) is a potent inhibitor of DNA polymerase γ , but only 3% of ddI is converted to this form. Furthermore, different cells have different phosphorylation capacities and this is dependent upon the state of the cell (active or resting). Cells also have varying amounts of mitochondria

(anywhere from thousands to none) depending on their cellular energy requirements. Therefore, it is inappropriate to extrapolate results from one cellular or tissue type to another. Finally, a special remark has to be made about 3TC: for this compound it was demonstrated that it not only has the capacity to inhibit the DNA polymerase activity of DNA polymerase γ , but it can also function as a substrate of the integral 3'-5'-exonuclease activity of this enzyme. The net result is that 3TC-TP is not likely to induce the termination of mtDNA replication [5]. Affinity for this exonuclease site has not been studied for other NRTI.

A better understanding of NRTI-induced mitochondrial toxicity will probably come from studies investigating the decrease in mtDNA content or an increase in lactic acid at clinically relevant concentrations [6,7]. In none of

these studies, however, are all NRTI tested with the same assay, impeding a fair comparison.

So far, the potential of the mitochondrial toxicity of NRTI has only been studied for each of the NRTI separately. Because it is clinical practice to use at least two NRTI in combination, studies on the feasible synergism of these agents in inducing this toxicity are urgently needed.

Clinical spectrum of mitochondrial dysfunction and nucleoside reverse transcriptase inhibitor toxicity

Mitochondrial disease has been studied extensively for many years in pediatrics and neurology [8]. Mitochondrial disease in these settings is usually caused by maternally inherited mutations or deletions of mtDNA genes. Theoretically, the complete depletion of all mtDNA genes by NRTI can potentially induce the complete spectrum of these symptoms, and indeed almost all the long-term side-effects of NRTI can be found within this spectrum. For most of these toxicities, the depletion of mtDNA or mitochondrial dysfunction has been demonstrated in the tissues involved [2•,9•,10]. In Table 1, the clinical symptoms of inherited mitochondrial dysfunction are listed, as well as observed NRTI-induced adverse events. Many of these symptoms have not been described as side-effects of NRTI, possibly because they have not been recognized (i.e. hypogonadism, diabetes, gastrointestinal pseudo-obstruction). Alternatively, many of these symptoms develop early in life, when inherited mitochondrial defects are already present. The observation of possible mitochondrial dysfunction months after perinatal exposure to NRTI might confirm this early development, because encephalopathy with seizures was among the leading symptoms in that study [11••]. In that retrospective study [11••], eight cases of possible mitochondrial dysfunction were observed in a cohort of 1754 mother-child pairs exposed to NRTI treatment during pregnancy. Two cases resembled Leigh’s syndrome and Alper’s syndrome with encephalopathy, persistent lactic acidosis, and defects in the mitochondrial respiratory chain complex. Lactic acidosis was observed in three other children. Although it remains unclear why these children developed these symptoms only after 4 months of NRTI exposure (and had therefore had time to replenish the eventually depleted mtDNA and mtDNA encoded proteins), the possibility of mitochondrial toxicity in neonates exposed to NRTI is of great concern and needs to be urgently studied.

Very limited data are available regarding the incidence of NRTI-induced mitochondrial dysfunction, either in children or adults. Prospective studies in large cohorts followed over an extended period of time will provide some of these answers. At present, a simple, non-

Table 1. Clinical features of mitochondrial dysfunction and adverse effects of nucleoside reverse transcriptase inhibitor treatment [2••,7]

Neuromuscular	External ophthalmoplegia and ptosis		
	Sensorineural deafness	ddC	
	Myopathy	ZDV	
	Migraine		
	Seizures/myoclonus		
	Encephalomyopathy	ZDV ^a , 3TC ^a	
	Dementia		
	Ataxia		
	Stroke-like episodes		
	Parkinsonism/dystonia		
	Spastic paraparesis		
	Peripheral neuropathy	d4T, ddl, ddC	
	Rhabdomyolysis		
Cardiac	Optic atrophy		
	Pigmentary retinopathy		
	Hypertrophic cardiomyopathy	ZDV, ddC, ddl	
Endocrine	Accessory pathways		
	Heart block		
	Diabetes mellitus	ddl	
Gastrointestinal	Hypoparathyroidism		
	Hypogonadism		
	Infertility		
Renal	Dysphagia		
	Cyclic vomiting		
	Pseudo-obstruction		
Haematological	Aminoaciduria		
	Renal tubular dysfunction	Adefovir =nucleotide RTI	
	Tubulo interstitial disease		
	Toni-Fanconi-Debre syndrome		
	Barrter syndrome		
	Sideroblastic anemia	ZDV	
	Pancytopenia	ZDV	
	Pancreas and liver	Exocrine pancreatic failure/pancreatitis	d4T, ddl
		Hepatocellular failure	ZDV, ddl, d4T
		Lactic acidosis	ZDV, ddl, d4T
Psychiatric	Depression		
	Psychotic illness		
Dermatological	Lipomatosis	d4T, all?	

^aObserved in children. ddC, zalcitabine; ddl, didanosine; d4T, stavudine; RTI, reverse transcriptase inhibitor; 3TC, lamivudine; ZDV, zidovudine.

invasive test is lacking, and to identify mitochondrial dysfunction the clinician therefore has to rely on the collection of clinical symptoms, which are often not very well defined.

Lactic acidosis

The most serious presentation of NRTI-induced mitochondrial dysfunction is lactic acidosis. Lactic acidosis can occur abruptly after months of NRTI therapy. It has been associated with ZDV, ddI and d4T, but is likely to occur with all NRTI. In most patients, the initial symptoms include nausea, vomiting, and abdominal pain, rapidly progressing to hyperventilation with severe acidosis, liver failure, and arrhythmia [12,13••]. Prospective data on the incidence are not available, but observational cohorts estimate the incidence at 1.3 per 1000 person-years [13••,14].

Cases spontaneously reported to the Food and Drug Administration include 46 cases of lactic acidosis from single NRTI treatment between 1988 and 1997, and 60 cases on double NRTI treatment from 1996 to 1998. In the later group d4T was involved in 46 out of 60 (76.7%) cases [15]. In an observational cohort from France [16] ($n=876$ with antiretroviral combination therapy, of which 532 contained d4T), 11 cases of symptomatic, mild hyperlactatemia were found, two of them with acute lactic acidosis. All 11 patients were receiving d4T at the time. After changing treatment to a regimen without NRTI, clinical improvement and the normalization of serum lactate was observed [16]. Similarly, Lonergan *et al.* [17] reported mild hyperlactatemia accompanied by abdominal pain and nausea in 10 patients treated on a d4T-containing regimen. The median duration of therapy was 10 months, with an estimated incidence of 20.9 cases per 1000 person-years. Six of the 10 patients had a liver biopsy, of which five showed macro- and microvesicular steatosis [17]. Although d4T appeared to be associated with lactic acidosis in those reports, the patients generally also had previous NRTI experience.

Fat redistribution/peripheral lipodystrophy syndrome

Morphological and metabolic complications associated with HAART are becoming increasingly evident and are of concern both to patients and clinicians. Persons afflicted with the fat redistribution/peripheral lipodystrophy syndrome may develop either dorsocervical lipoma, central adiposity, breast hypertrophy/gynecomastia, peripheral fat loss (lipoatrophy), or a combination of those symptoms. Hyperlipidemia or insulin resistance may also be present [18^{••},19]. Other authors have noted myopathy and hepatic steatosis with lactic acidosis [20] as accompanying features [21^{••}]. Originally associated with protease inhibitors (PI), it is now evident that this condition can also occur in persons who have never been exposed to these drugs but who have received two NRTI [22[•],23^{••},24–26]. Observational cohort studies [21^{••},23^{••},27–30] have further demonstrated a significant relationship between the development of lipodystrophy (especially lipoatrophy) with the duration of NRTI exposure, particularly with the use of d4T. Preliminary studies of switching from d4T to ZDV have shown some improvement of the body habitus changes [31[•],32[•]].

Non-HIV related lipodystrophies include partial and generalized lipodystrophy, and Madelung's disease (benign or multiple symmetric lipomatosis). Several clinicians have noted a physical similarity between Madelung's disease and lipodystrophy associated with HAART [20,33[•]]. Interestingly, the etiology of Madelung's disease is associated with chronic alcoholism, but

also with inherited mitochondrial dysfunction [34–36]. The formation of dorsocervical lipomas in these patients is associated with the A8344G mutation in mtDNA [37,38]. Insulin resistance and diabetes can also be attributed to mitochondrial dysfunction [39–41].

On the basis of these observations, we independently hypothesized that NRTI may cause fat redistribution as a result of NRTI-induced mitochondrial toxicity (T.N.) [3^{••},42^{••}]. Our hypothesis does not exclude the possible role of PI in the syndrome: both classes of drugs may act complementarily in its etiology. Mallal *et al.* [28] demonstrated an increased rate of fat wasting after the addition of PI to a double NRTI regimen. In this respect, one may speculate as to the possible involvement of a drug–drug interaction; for example, indinavir elevates d4T exposure (as measured by the area under the plasma concentration time curve) by $25 \pm 26\%$. Further studies are required to evaluate the individual roles of the drugs in this complex syndrome.

Emerging risk factors

Several independent risk factors can influence NRTI-induced mitochondrial toxicity; these are summarized in Table 2. Of particular concern is hydroxyurea, a drug used to potentiate the activity of ddI, adefovir, and other adenosine analogues [43]. Reports of adverse effects involving ddI and hydroxyurea include the increased incidence of peripheral neuropathy, neurotoxicity, fatal hepatitis, and prolonged bone marrow suppression [44,45[•],46[•],47]. Fatal pancreatitis has also been noted. These observations underscore the narrow therapeutic index for ddI and other NRTI (e.g. adefovir, lodenosine, and d4T); drugs that boost NRTI activity or alter its pharmacokinetic disposition may increase the likelihood or severity of toxicity. Other drugs such as alcohol, high-dose tetracycline, and valproic acid are known mitochondrial toxins [48,49]. The concomitant use of these drugs may exacerbate NRTI-induced mitochondrial toxicity. Finally, hepatic mitochondrial damage has been demon-

Table 2. Risk factors for nucleoside reverse transcriptase inhibitor-induced mitochondrial toxicity

Alcohol
Concomitant medications
Amiodarone
Aspirin
Chloramphenicol
Hydroxyurea
Non-steroidal anti-inflammatory agents
Tetracycline
Valproic acid
Genetics
Hepatitis C
Multiple NRTI use
Nutritional status
Prolonged NRTI exposure

strated in patients with chronic hepatitis C virus (HCV) infection [50*]. This observation has led to the hypothesis that HCV-induced hepatitis may be mediated by the mitochondria [50,51*].

Management options

Mitochondrial toxicity, in general, is reversible upon discontinuation of the NRTI. Myopathy, bone marrow suppression and, to a lesser extent, peripheral neuropathy will resolve in this manner. These side-effects tend to re-occur after rechallenge. Alternative options include dose reduction or continuation of treatment despite the adverse effects. For more severe symptoms, such as lactic acidosis, NRTI should be stopped immediately. Scarce data are available as to whether rechallenging with alternative NRTI is feasible in these cases: in one patient lactic acidosis immediately re-occurred after alternative NRTI were tried [52].

Pharmacological treatment of mitochondrial dysfunction is currently based upon anecdotal experience and knowledge of the biochemical and enzymatic pathways. Table 3 provides a list of agents that have been tried in inherited mitochondrial diseases with varying success. For the most part, clinical endpoint studies have not been conducted with these agents, and the dosages provided are empiric and are not based on dose-ranging studies. Furthermore, very few of these agents have been used in the context of NRTI-induced mitochondrial toxicity. Vitamins C (ascorbic acid) and E (α -tocopherol) improved ZDV-induced myopathy *in vitro* [53]. L-Carnitine or acetyl-carnitine has been suggested for NRTI-induced myopathy and peripheral neuropathy because these patients often have carnitine depletion [54,55]. In three reported cases of lactic acidosis, riboflavin (vitamin B₂) [56,57] and ubiquinone (coenzyme Q₁₀) [58] were suggested to improve the clinical condition.

Table 3. Agents tried for treatment of mitochondrial dysfunction

α -Lipoic acid	10 mg/kg/day
Biotin	0.05 mg/day or twice a day
L-Carnitine	50–200 mg/kg/day
Coenzyme Q ₁₀ (ubiquinone)	3.5–4 mg/kg/day
Folate	0.4 mg/day or twice a day
N-acetyl-cysteine	200 mg/kg/day
Pantothenic acid	50 mg/day or twice a day
Vitamin B ₁ (thiamine)	50–300 mg/day or twice a day
Vitamin B ₂ (riboflavin)	30–400 mg/day or twice a day
Vitamin B ₃ (niacin)	50 mg/day or twice a day
Vitamin B ₆ (pyridoxine)	50 mg/day or twice a day
Vitamin B ₁₂ (cyanocobalamin)	0.05 mg/day or twice a day
Vitamin C	25 mg/kg/day
Vitamin E	25 IU/kg/day
Vitamin K ₃ (menadione)	20–500 mg/day
Zinc picolinate	30 mg/day or twice a day

Dosages are arbitrary and have not been subjected to controlled trials.

On the basis of these observations, there is a rationale to initiate studies for the evaluation of the possible beneficial effects of any of these agents in the treatment and prevention of NRTI-induced mitochondrial toxicities. Until such studies are performed, it is reasonable to treat NRTI-induced lactic acidosis with a combination of riboflavin, ubiquinone, L-carnitine, and thiamine [13**].

Conclusion

NRTI clearly play a central role in the treatment of HIV. However, the prolonged and combined effect of these drugs on the mitochondria is largely unknown. Hepatic steatosis and lactic acidosis are consequences of mitochondrial dysfunction; we speculate that lipodystrophy may also be related to impairment of the mitochondria. Several risk factors may be involved in the development of mitochondrial toxicity, and the optimal management of this condition has not been determined. Therefore, investigations into the effects of NRTI on mitochondria are necessary to determine the importance of this toxicity and to develop strategies for its prevention and treatment.

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