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Mitochondrial Toxicity of Nucleoside Analogue Reverse Trancriptase Inhibitors: Lactic Acidosis, Risk Factors and Therapeutic Options

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Abstract

Nucleoside analogue reverse trancriptase inhibitors (NRTIs) inhibit also the function of DNA polymerase γ, the only enzyme responsible for the replication of mitochondrial DNA (mtDNA). The resulting mitochondrial dysfunction leads to similar clinical features as can be observed in inherited mitochondrial diseases. The most threatening event is (fatal) lactic acidosis, which will be discussed in more detail. In this review we analyse the possible risk factors for the development of NRTI- related mitochondrial dysfunction and consider possibilities for therapeutic and preventive treatment.

Key words

Nucleoside analogue reverse trancriptase inhibitors. NRTIs. Adverse effects. DNA polymerase γ. Mitochondrial toxicity. Lactic acidosis. Risk factors. Treatment.

Introduction

During the last fifteen years, major progress has been made in the understanding and treatment of HIV infection. Although the initial treatment of the infection with monotherapy of zidovudine (AZT) turned out to be only shortly successful, the current guidelines advise a combination of antiretroviral drugs, which enables suppression of viral replication so efficiently, that immunological recovery has become feasible. As long as this viral suppression is maintained, it seems possible now to extend the life expectancy of HIV-infected individuals for several years. Unfortunately, lasting viral suppression can only be achieved by continuous administration of the antiretroviral drugs. Besides the fact, that 100% adherence to the stringent medication schedules is a demanding task psychologically, side-effects of the medication further hampers long-term therapy.

In this review, we will discuss the toxicity of one class of antiretroviral drugs, the nucleoside analogue reverse transcriptase inhibitors (NRTIs). Since we recently reviewed the literature, demonstrating that the common pathway of NRTI toxicity is an induced mitochondrial dysfunction[1], herein we will focus on lactic acidosis, risk factors and therapeutical guidelines.

DNA polymerases and NRTIs

After NRTIs are tri-phosphorylated intracellularly to nucleotides, they are incorporated in the growing DNA chain by the viral enzyme reverse transcriptase, which prohibits further DNA elongation (NRTIs lack the critical hydroxyl group at the 3'-position of the sugar residue of the nucleotide). Also other enzymes, capable of DNA formation (DNA-polymerases), can theoretically use these nucleotides as substrate and indeed, of the five known human DNA polymerases, both DNA polymerase β and γ

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have a high affinity for these compounds[1-10]. The only exception for the inhibition of DNA polymerase γ is possibly lamivudine (3TC), which at this moment is the only NRTI that acts as inhibitor of the polymerase activity and concomitantly as substrate of the integral 3'-5' exonuclease activity of this enzyme: incorporation is immediately followed by excision, enabling continuation of the DNA chain elongation[7].

DNA polymerase β is involved in repair of nuclear DNA. So far, no clinical effects have been reported, that were related to inhibition of this enzyme by NRTIs, but one might envision that cancer incidence will rise in people on long-term NRTIs, due to an ineffective DNA repair mechanism.

DNA polymerase γ is the only enzyme responsible for the replication of mitochondrial DNA (mtDNA) and the inhibition of this enzyme by NRTIs can easily lead to a depletion of mtDNA[1,11-13], also leading to depletion of mtDNA encoded proteins. Among these proteins are 13 subunits of the oxidative phosphorylation (OXPHOS) system[1,14] and their down regulation will definitely lead to an impaired energy production of the cell (Fig. 1). Many hereditary mutations or deletions in the mtDNA have been described, causing a heterogeneous spectrum of characteristic clinical features (Table 1)[15-19], the down regulation of the complete mtDNA by NRTIs can lead to the complete spectrum of these symptoms[1,20].

Table 1. *Clinical features of mytochondrial dysfunction in relation to NRTI adverse reactions.*

Clinical features:	observed as adverse event for:
Neuromuscular:	
polyneuropathy	ddC, ddI, d4T
myopathy	AZT
Cardiomyopathy	AZT, ddC, ddI
Hepatocellular:	
steatosis, lactic acidosis	AZT, ddI, d4T
Gastrointestinal:	
pancreatitis	ddI, d4T
Hematological:	
pancytopenias	AZT
Nephrological:	
proximal tubular dysfunction	ADV

ddC = zalcitabine, ddI = didanosine, d4T = stavudine, AZT = zidovudine, ADV = adefovir (nucleotide analogue)

Mitochondrial dysfunction and NRTI toxicity

Nearly all adverse effects that have been attributed to the use of NRTIs can indeed be found among the list of clinical symptoms, that have been described for hereditary mitochondrial dysfunction (Table 1)[1,20]. Also the proximal tubular defect found during treatment with the nucleotide analogue RTI adefovir[21], is believed to be caused by impaired mi-

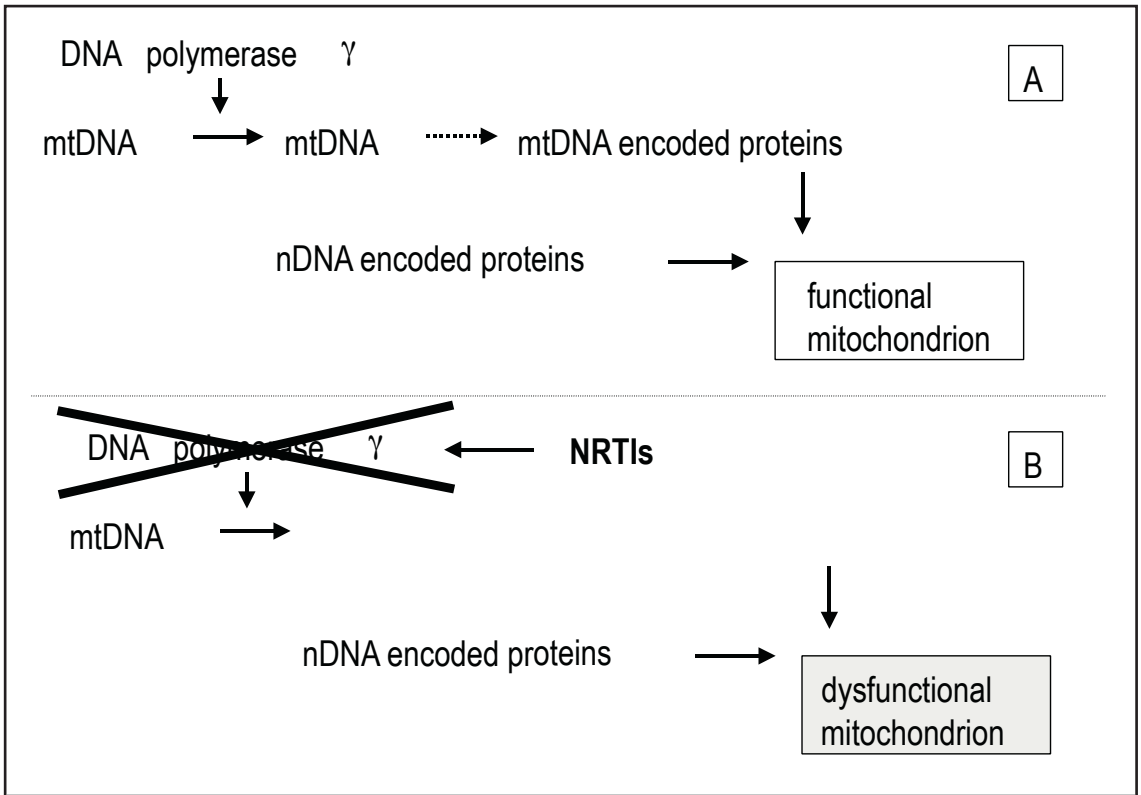

Fig. 1. *Schematical representation of the necessary components for normal mitochondrial function (panel A). Most of the mitochondrial proteins are encoded by nuclear DNA (nDNA), 5-10% is encoded by mitochondrial DNA (mtDNA). mtDNA replication is regulated by the nuclear encoded enzyme DNA polymerase γ. NRTIs inhibit the function of DNA polymerase γ (panel B), leading to depletion of mtDNA encoded proteins and mitochondrial dysfunction.*

tochondrial function[1], since also adefovir demonstrates high affinity for DNA polymerase γ[22].

Direct proof for NRTI induced mitochondrial toxicity was demonstrated in zidovudine (AZT) related myopathy in humans[23-27] and zalcitabine (ddC) related neuropathy in rats[28,29]. Recently, in a patient with massive liver steatosis and lactic acidosis, due to AZT treatment, also depletion of mtDNA in skeletal muscle and liver tissue was found[30]. The biochemical abnormalities found in NRTI related lactic acidosis, as well as the acquired L-carnitine deficiency observed during d4T, AZT or adefovir treatment[31,32], form an indirect evidence for mitochondrial failure (see below).

Except for the AZT related myopathy, no clinical studies have been done to definitely demonstrate mitochondrial dysfunction during the other NRTI related side-effects. One of the main problems for those studies in the future will be the absence of a reliable, non-invasive test. The only assay so far, that definitely demonstrates mitochondrial dysfunction, is a tissue biopsy, in which the several oxidative phosphorylation steps can be tested biochemically and the mtDNA content can be quantified.

A striking feature of the NRTI toxicity is its apparent tissue specificity: Myopathy can be caused by AZT, but hardly by any of the other NRTIs and conversely, neuropathy and pancreatitis are common features in treatment with ddC, didanosine (ddI) and stavudine (d4T), but not with the other NRTIs[1]. In one study, in a patient with AZT induced lactic acidosis and myopathy, no mtDNA depletion could be found in myocardium or kidney, while this was clearly demonstrated in the liver and muscle tissue[30]. Furthermore, no mtDNA depletion could be demonstrated in brain tissue of AZT treated patients[33]. A possible explanation for this phenomenon is the so-called '*pol*-γ' hypothesis (Table 2)[1,12,20]. In this hypothesis four factors contribute to an effective inhibition of DNA polymerase γ by a certain NRTI at a special tissue level, if 1) this NRTI has the pharmacodynamic capability to enter the target cells, if 2) the target cell possesses the right cellular nucleoside kinases to monophosphorylate and later on triphosphorylate the NRTI, if 3) the triphosphorylated NRTI can inhibit DNA polymerase-γ either by serving as a competitive (ineffective) alternate substrate or by chain termination of the nascent mtDNA strand (non-competitive), and if 4) the target tissue has a metabolic reliance on oxidative phosphorylation[12,20].

Lactic acidosis and hepatic failure

The most severe of all NRTI toxicity's is lactic acidosis, which is in most cases fatal. It has been described for AZT[30,34-37], ddI[37-39] and d4T[40-43]. The incidence is estimated around 1.3 per 1000 person-years, retrospectively found in a cohort of antiretroviral users[44]. This number fits reasonably well with our own experience, when we found 4 fatal cases within one year in The Netherlands, where around 3000 patients are treated with antiretroviral combination therapy (ter Hofstede *et al.*, submitted for publication).

Table 2 . *Polymerase γ hypothesis[12,20141].*

1. NRTI has to cross the target cell membrane (and the mitochondrial membrane).
2. NRTI needs to be (tri)phosphorylated by cellular kinases of the target cell.
3. The tri-phosphorylated NRTI inhibits DNA polymerase γ.
4. The metabolism of the target tissue relies importantly on oxidative phosphorylation.

The clinical course is characterised by an episode of malaise, nausea and vomiting, often accompanied by abdominal pain and hyperventilation (compensatory for the acidosis), finally resulting in rapid liver failure and uncontrollable arrhythmia's (Table 3). Biochemically, lactic acidemia is found, but more importantly, also an increase in the ratio's of lactate/pyruvate and ß-hydroxybutyrate/acetoacetate. Table 4 shows an example of these values, in a female patient, who developed lactic acidosis 7 months after she started a combination of d4T/3TC/saquinavir[41]. She was admitted with profuse vomiting, for which there was no explanation after endoscopic and neurologic examination. She started to hyperventilate on the 11th day of admission, shortly after enteric tube-feeding was started. She deteriorated rapidly and died 3 weeks later, despite bicarbonate dialysis and intensive care support. Autopsy revealed severe hepatomegaly with microscopically marked cholestasis and moderate, pericentral, micro- and macrovesicular steatosis. Electro-microscopy showed no striking abnormalities at the mitochondrial level, but southern blot analysis of mtDNA gave no signal, probably due to complete mtDNA depletion.

Table 3. *Clinical symptoms of NRTI-induced lactic acidosis.*

- nausea
- vomiting
- abdominal pain
- hyperventilation
- liver failure
- arrhythmia

Table 4. *Example of biochemical deterioration in patient with NRTI induced lactic acidosis.*

	Day 11 of admission	Day 12	Reference value
Lactate (L)	9.8	19.9	< 0.8 mmol/L
Pyruvate (P)	0.3	0.4	< 0.07 mmol/L
L/P ratio	33	50	< 18
3HB/AA ratio	3.0	3.5	< 2

3HB = 3-hydroxy-butyrate, AA = aceto-acetate

The only explanation for the biochemical abnormalities can be found in mitochondrial failure (Fig. 2): A disturbed function of the OXPHOS-system will give rise to a disturbed redox state (increased NADH/NAD+ ratio), which will shift the pyruvate/lac-

tate equilibrium in the direction of lactate. Consequently, both lactate, leading to lactic acidemia or even lactic acidosis, as well as the lactate/pyruvate ratio increase. This is particularly true in the post-absorptive period, when more NAD+ for the adequate metabolism of glycolytic substrates is needed[18]. Similarly, a postprandial increase of ketone bodies synthesis can be observed, related to the channelling of acetyl-CoA toward ketogenesis[45]. Fat (triglycerides and free fatty acids) will accumulate intracellularly, which can be demonstrated histologically (macrovesicular hepatic steatosis). In the patient described above, the only abnormal biochemical finding on admission, was an sudden rise of triglycerides (from 2,3 to 8,1 mmol/L).

Therapeutical considerations for mitochondrial failure will be described below, but it is important to stress here, that in cases of lactic acidemia, high amounts of glucose should be avoided, since this can rapidly lead to uncontrollable deterioration.

Risk factors

Mitochondrial dysfunction develops when the energy-generating capacity falls below a certain energetic threshold[15,17,20,46,47]. One can envision, that as long as the energy-generating capacity remains above the minimal energy requirement, no dysfunction will be noticed, although the energy-generating capacity might already be lower than the maximum possible (Fig. 3). Many factors can influence this process in the cell[48].

At first, structural mtDNA defects cause impaired mitochondrial function and decreased energy production. Apart from the wellknown hereditary mtDNA defects[19,49] (Fig. 3B), there is a significant mtDNA polymorphism in the general population[14,15,47], which even increases during ageing[17] (Fig. 3A). Secondly, depletion of the exogenous factors in the OXPHOS system, like vitamins and other co-factors, will negatively influence the oxidative phosphorylation system and its energy-generating capacity (Fig. 3)[48]. Those situations can be seen in malnourished patients or in patients who are just recovering from serious infections or inflammatory processes, like pancreatitis. Thirdly, tissues with a higher energy demand, have a higher minimal energy requirement and will develop more easily energy deficits[14,50]. Furthermore, some endocrine disorders, toxic agents (other than NRTIs, like alco-

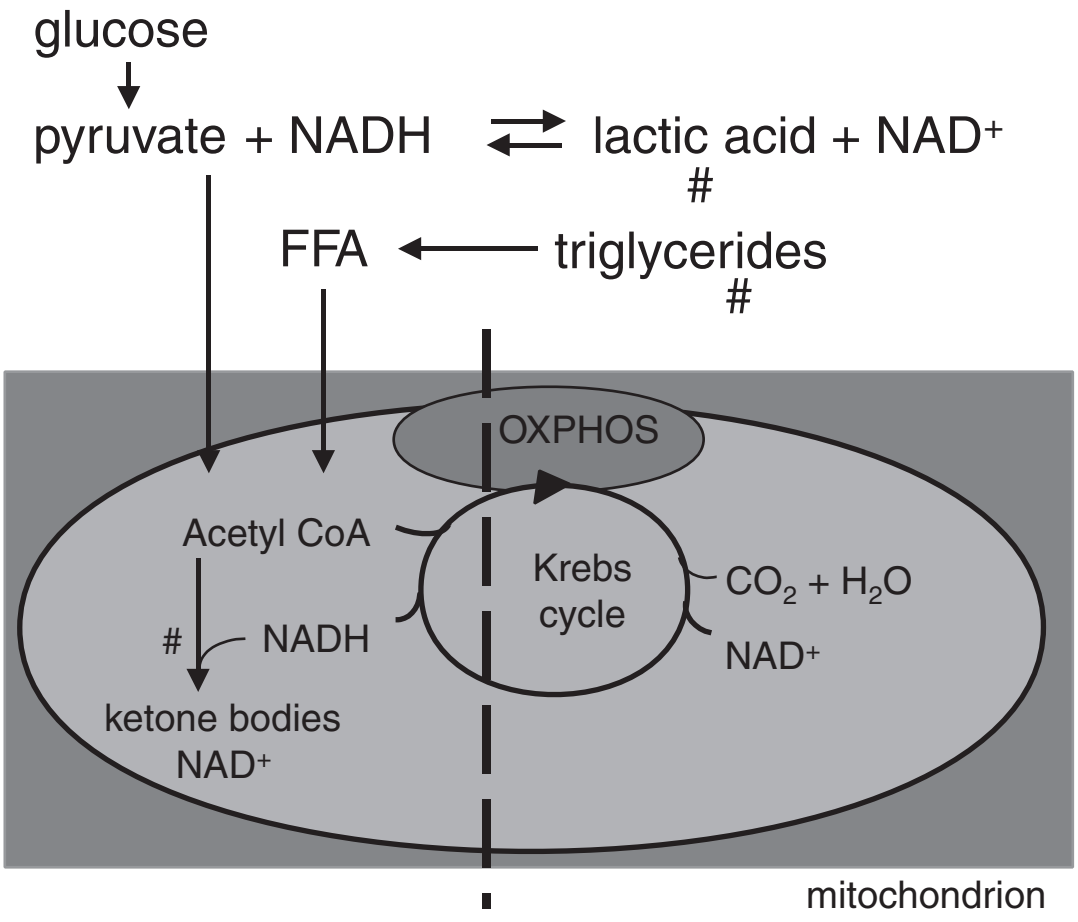

Fig. 2. *Schematic representation of the glucose metabolism and the role of the oxidative phosphorylation system (OXPHOS) within the mitochondrion. Blocking OXPHOS (dotted line) will lead to an accumulation and generation of lactic acid, free fatty acids (FFA), triglycerides and ketone bodies (marked by #).*

hol, acetaldehyde, chloramphenicol and tetracyclines) and other extramitochondrial inborn errors of metabolism, can negatively influence mitochondrial function[48]. All these factors can interact one another synergistically.

In persons with a lower energy-generating capacity, mitochondrial toxicity of NRTIs will sooner lead to energy deficits than in persons with higher ones (Fig. 3C). Therefore, all factors mentioned above might explain the observed inter-individual variation of NRTI toxicity's: Some patients develop adverse reactions to almost all NRTIs, while others hardly suffer any symptoms on long-term treatment.

Apart from these factors, polymorphism of DNA polymerase-γ might also display different affinities of the NRTIs for this enzyme, which will lead to different individual susceptibilities for NRTI inhibition. So far, no data are available on DNA polymerase-γ polymorphism, but polymorphism has been described for instance for DNA polymerase-β[51].

Finally, the development of mitochondrial dysfunction is a time-dependent process. All adverse events of the NRTIs, enlisted in Table 1, develop only after weeks to months of exposure, possibly accelerated by the environmental factors, mentioned above. Furthermore, a combination of NRTIs might synergistically lead to increased inhibition of mtDNA replication, and therefore to an increased toxicity[37]. The addition of hydroxyurea, which appears to enhance the antiviral effects of (some) NRTIs[52,53], might further aggravate the already existing mitochondrial toxicity, as was recently found for toxic hepatitis[54].

Reversibility and treatment options

In contrast to inherited mtDNA defects, acquired mtDNA deletions (and thus the mitochondrial dysfunction) might be restored, when the inducing agents, the NRTIs, are removed. There are no data about kinetics of mtDNA replenishment after interruption of NRTIs, nor about the recuperation of mitochondrial function in these situations. Looking to the recovery of bone-marrow after the cessation of AZT in patients with AZT induced anaemia (or leucopenia), one has to conclude that mitochondrial toxicity can be reversed completely and rapidly. Some tissues, however, seem to recover only very slowly; NRTI induced neuropathy can last for months after NRTIs have been stopped. The factors that determine this recuperation capacity are unknown, but tissues with a high cell turnover (like bone-marrow) seem to have an advantage over cells that divide slowly (like neurons), as if tissue function can only be restored by renewal of cells. The healing of AZT induced myopathy[55] is at variance with this view, but the fact that hepatic steatosis (and in some cases also lactic acidosis[37,40,42,43]) can disappear rapidly, supports it. Apparently, both cell vulnerability and cell division capacity seem to play a role, but no data are available to further clarify this issue.

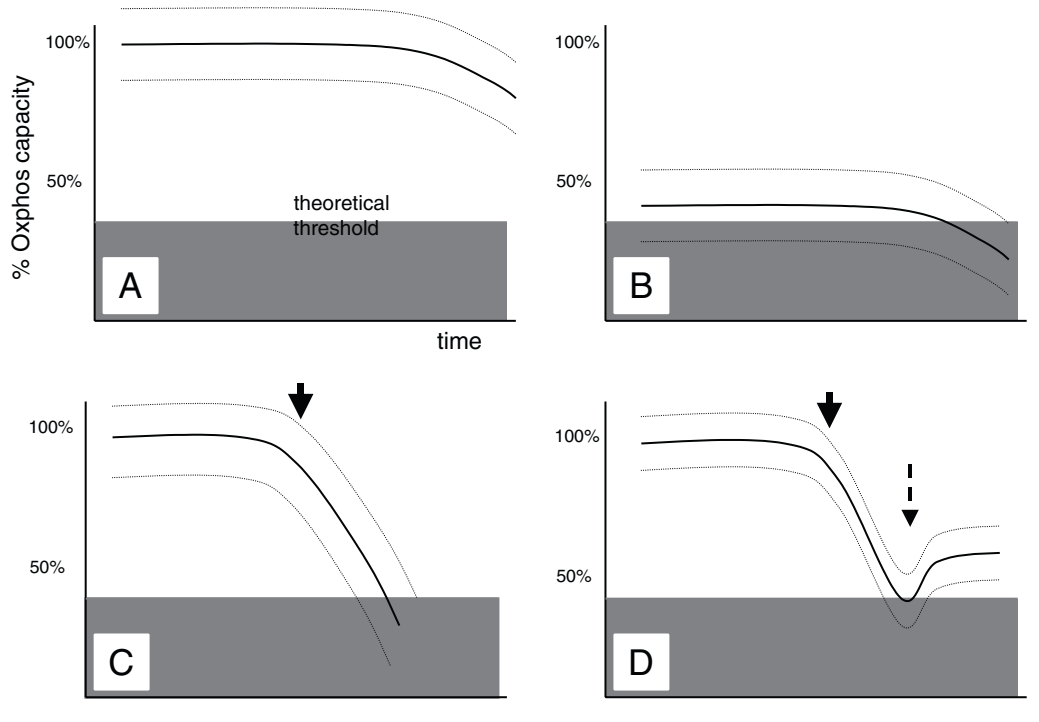

Fig. 3. *Theoretical graphs, depicting the energy generating capacities of OXPHOS during several conditions. In the normal situation (panel A) the OXPHOS capacity is set at an average of 100%, but within the general population there is a considerable standard deviation (dotted lines). During ageing, the OXPHOS capacity drops in every individual, due to acquired mtDNA mutations and deletions[17]. Inherited mtDNA defects lead to diminished OXPHOS capacity (panel B), but treatment with NRTI (arrow) will also decrease this capacity (panel C). Due to the standard deviation, not every individual will reach the threshold at the same time-point. The suppletion of essential mitochondrial co-factors (dotted arrow) might improve the OXPHOS capacity to some extent (panel D).*

Suppletion of essential co-factors (e.g. thiamine, riboflavin and L-carnitine), artificial electron acceptors (vitamin C and K_3) and anti-oxidants [coenzyme Q_{10} (CoQ_{10}), L-carnitine, and α-tocopherol (vitamin E)] has been tried in congenital mitochondrial diseases with varying success[56-63]. Therefore, these substances might be beneficial in NRTI mitochondrial diseases as well, as was already demonstrated with vitamin C and E at supranutritional dosages in AZT treated mice[64]. Recently, a few case reports have been published, in which treatment with either riboflavin (50 mg daily)[42,43] or CoQ_{10} (dosage not given)[40] seemed to induce the recovery of NRTI related lactic acidosis. In the last case[40], NRTI treatment was stopped as well, but in the first two cases, NRTI interruption was not mentioned[42,43]. As we have outlined above, we believe cessation of NRTI therapy is essential in these cases. *In vitro*, L-carnitine both prevented and improved AZT induced myopathy[65,66]. Accumulation of acyl-CoA in mitochondrial dysfunction (see above) causes L-carnitine deficiency[48] and in fact this is exactly what was observed in patients suffering NRTI induced neuropathy[31], but also in adefovir (= nucleotide analogue RTI) induced nephropathy[32].

Based on these results, there seems to be a rationale not only to treat, but also to prevent NRTI induced mitochondrial toxicity's with suppletion of L-carnitine[67], ribiflavin, CoQ_{10} and others. Placebo-controlled, randomized studies need to be done, in order to demonstrate the beneficial effects of these compounds and the minimal effective dosages.

As long as definite data are missing, in cases of lactic acidosis, one should give these supplements and add not only riboflavin, but also thiamine, CoQ_{10} and L-carnitine. The dosages used for these supplements have had a wide variation and were seldomly based on dose-effect studies[56]. Dosage recommendation is therefore arbitrary (Table 5) and should also be subjected to controlled studies.

Table 5. *Possible therapy in NRTI-mitochondrial dysfunction[56-62].*

Substance	Daily dose range
thiamine (vitamin B1)	0.5 – 300 m
riboflavin (vitamin B2)	30 – 400 mg
vitamin C	250 – 4000 mg
α-tocopherol (vitamin E)	200 – 400 IU
vitamin K_3	20 – 500 mg
coenzyme Q_{10}	60 – 300 mg
L-carnitine	50 – 200 mg/kg

Conclusions

NRTI induced mitochondrial dysfunction is the basic pathogenetic event in the development of NRTI related toxicity's, but several contributing factors might aggravate the symptoms. The generation of NRTI induced mitochondrial dysfunction is a time-dependent process and symptoms develop gradually.

Especially in cases of lactic acidosis, early recognition is essential, since symptoms become rapidly irreversible. NRTI treatment should be stopped immediately and the addition of substances, like thiamine, riboflavin, CoQ and L-carnitine should be considered.

These substances might also play a role in postponing, preventing or even treatment of the other NRTI related toxicity's.

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