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Update on Mitochondrial Toxicity of Antiretrovirals and its Link to Lipodystrophy

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Abstract

The dramatic improvement seen in the latest years in the prognosis of HIV infection has been threatened by long-term toxicities of antiretroviral drugs. Nucleoside analogs remain the cornerstone of antiretroviral therapy, but these compounds seem to produce mitochondrial damage leading to a broad range of side effects, which depend of the organ/tissue affected. Among those toxicities are of particular concern lipoatrophy and hyperlactatemia syndromes. This review will focus on the pathogenesis of mitochondrion damage caused by nucleoside analogs and its clinical consequences, particularly in respect to body-shape changes.

Key words

Mitochondrial toxicity. Hyperlactatemia. Lipodystrophy. Metabolic complications.

Recent years have witnessed an impressive decrease in mortality and disease progression in HIV-infected patients[1], but these significant advances have been threatened by long-term toxicities of antiretrovirals. Several metabolic complications have emerged as distressing issues for both HIV-infected subjects and their providers. This review will focus on two of these complications-hyperlactatemia syndromes as well as lipodystrophy, both of which have been linked to mitochondrial dysfunction.

Mechanisms of NRTI-induced mitochondrial toxicity

The most important and critical mitochondrial function is oxidative phosphorylation, a mechanism by which the energy derived from metabolism of nutrients in the presence of oxygen is transformed into adenosine triphosphate (ATP) (Fig. 1). Each cell contains hundreds to thousands of mitochondria, with each mitochondrion containing 2 to 10 mtDNA molecules[2]. Mitochondrial DNA is prone to mutations at a 5- to 10- fold higher rate than nuclear DNA; a mixture of wild-type and mutant mtDNA can coexist within the same cell, a condition known as heteroplasmy. The consequences of such mutations will depend on the proportion of normally functioning and abnormally functioning mtDNA in a particular cell. Once the proportion of abnormal mtDNA exceeds a certain threshold level, cellular function is impaired. The threshold of mutated genome needed to produce a deleterious phenotype varies among persons, among organ systems, and within a given tissue.

Inherited disorders of mitochondrial function or biogenesis exhibit several of the features of the nucleoside reverse transcriptase inhibitor (NRTI)-associated toxicities, including liver steatosis, lactic acidosis, and lipodystrophy[3]. Although mitochondrial dysfunction has been considered the culprit in most NRTI-associated toxicities[4], as shown in figure 2, the exact mechanism(s) remains under investigation. NRTI are capable of inhibiting

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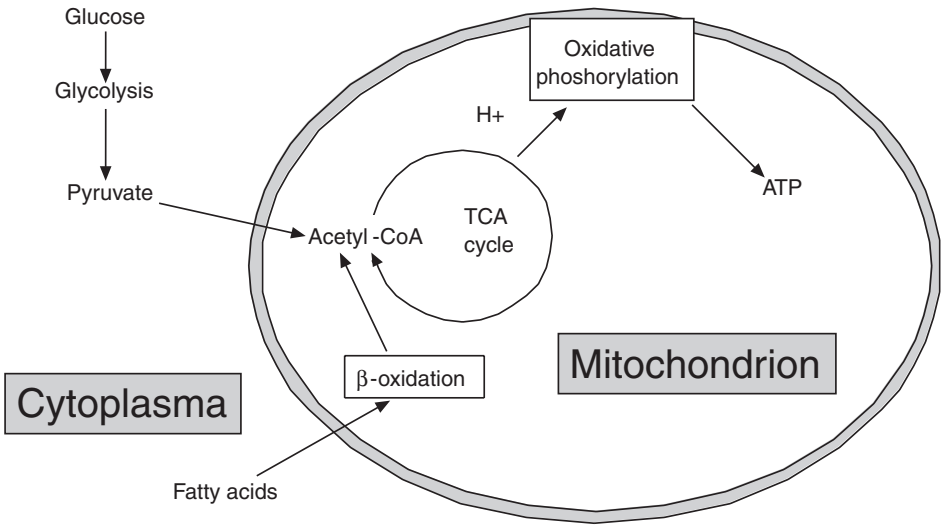

Figure 1. *Schematic representation of the steps of oxidative adenosine triphosphate (ATP) energy production in the mitochondria. After formation by glycolysis, pyruvate is metabolized by pyruvate dehydrogenase to acetyl coenzyme A (Acetyl CoA). Acetyl CoA donates its acetyl group to the first compound of the Krebs cycle, oxaloacetate, to form citrate, beginning a turn of the oxidation cascade of the krebs cycle. These reactions yield CO_2 and H+, the latter of which is trapped in the form of reduced coenzymes, which transfers its reducing equivalents to the first complex of the respiratory chain, with subsequent flow of reducing equivalents, electrons, in the respiratory chain. This will reduce oxygen to water; the resultant proton gradient is sufficient for synthesis of ATP.*

Symptom	NRTI Involved
Lactic acidosis	d4T, ZDV, ddI
Pancreatitis	ddI, d4T, 3TC in children
Neuropathy	ddC, ddI, d4T
Myopathy	ZDV
Cardiomyopathy	ZDV
Lipodystrophy	d4T > other NRTI

Figure 2. *Clinical adverse effects associated with NRTI use*

mtDNA synthesis through inhibition of DNA polymerase gamma, the primitive DNA polymerase found in mitochondria[4-9]. This mtDNA depletion may worsen after long exposure to NRTI to a point at which a threshold of energy depletion may be reached and symptoms become manifested. The degree of DNA polymerase gamma inhibition, and subsequently of mtDNA depletion depends on the type of nucleosides used (ddC > d4T > ddI = AZT > 3TC > abacavir)[5,9]. Recent data showed that tenofovir is unlikely to induce significant mtDNA depletion[10].

Other investigations pointed to the role of reactive oxygen species in the generation of NRTI-associated mitochondrial dysfunction. Zidovudine has been shown to increase mtDNA oxidized guanosine levels[11,12]. A recent study showed a beneficial role of antioxidants in reversing zidovudine-induced histological mitochondrial damage[13]. Another study demonstrated that antioxidants were able to reverse d4T-induced oxidative stress in mice[14]. Ongoing studies are exploring the role of antioxidants for treatment as well as prevention of NRTI-induced mitochondrial toxicity. Other possible mechanisms of mitochondrial dysfunction include mtDNA deletions, which have been shown in liver[15], muscle[16], fat[16], and sperm[17] of NRTI-treated subjects with presumed NRTI-related toxi-

cities. Finally, NRTI may also be associated with uncoupling of the electron transport chain from ATP synthesis or with direct inhibition of mitochondrial enzymes[18].

Lactic acidosis and symptomatic hyperlactatemia

Lactic acidosis has been reported in HIV-infected patients since the early 1990s, but recent clusters of reports increased physicians' awareness about this potentially fatal syndrome. Fortunately, it remains a rare occurrence. The typical clinical presentation is one of precipitous occurrence of nausea, vomiting, abdominal pain, fatigue, malaise, tachycardia, dyspnea on exertion, rapid weight loss, hepatic steatosis, and at times hepatic failure[19-21]. Observational cohort studies suggest a likely incidence of 1.3-1.7 cases per 1000 person-year of NRTI exposure[22-24].

More common than the severe lactic acidosis, is a milder form of mitochondrial dysfunction termed symptomatic hyperlactatemia. Its incidence has been reported at 25.6 cases per 1000 person-years among d4T users, compared with 1.9 cases per 1000 person-years among other NRTI users[25]. In a recent report, Brinkman estimated the incidence of clinically significant hyperlactatemia at 11 per 1000 person-years on antiretroviral therapy[26]. Stavudine has been involved in most of these cases[25,27-29]. Female gender and obesity are risk factors for both lactic acidosis and the milder symptomatic hyperlactatemia[30,31]. In addition, HIV-infected pregnant women treated with the combination of d4T and ddI seem to be at particular risk of lactic acidosis with or without pancreatitis[32,33]. The risk appears to be greatest in the third trimester and with longer duration of ddI/d4T therapy. Therefore, the combination use of d4T/ddI should be avoided in HIV-infected pregnant women, except in cases of very limited treatment options in which the potential benefits clearly outweigh the risks of these drugs[33]. In addition, NRTI-treated pregnant women deserve close observation with a low threshold for lactate and lactate/pyruvate testing in case of any suggestive symptoms. There have been no demonstrated correlations between these cases and CD4+ cell count, duration of HIV infection, clinical stage of disease, HIV-1 RNA levels, and use of PI or NNRTI therapy[30]. Clinical symptoms associated with symptomatic hyperlactatemia include fatigue, weakness, nausea, abdominal pain, weight loss, or otherwise unexplained increased liver transaminases. It is usually associated with hepatic steatosis, and at times with myopathy and pancreatitis. The FDA recently reported 25 cases of profound motor weakness suggestive of Guillain-Barré syndrome, all associated with hyperlactatemia[33]. Twelve of the cases occurred in women, and six of the seven fatalities occurred in women. This is in keeping with prior reports of lactic acidosis in which female gender was overrepresented[30,31].

Hepatitis C itself is associated with lipoperoxidation that can lead to mitochondrial dysfunction and depletion in mtDNA[34]. A recent study found significant mtDNA depletion as well as electron microscopic alterations of mitochondria in 92% of patients with hepatitis C, genotype 1[35]. In addition, ribavirin may enhance the risk of NRTI-associated mitochondrial dysfunction[36]. Therefore, in subjects co-infected with HIV and hepatitis C, the potential for mitochondrial damage is significant; these subjects deserve close observation and a low threshold to undergo mitochondrial testing in case of any suggestive symptom(s).

Diagnosis of lactic acidosis / symptomatic hyperlactatemia

Ultrastructural and functional defects in liver and muscle mitochondria have been demonstrated in NRTI-treated patients with symptomatic hyperlactatemia or lactic acidosis[25,29,37-39]. Other than elevated serum lactate levels, other laboratory abnormalities reported in these patients included elevated liver and pancreatic enzymes, elevated lactate/pyruvate ratios, and elevations in muscle enzymes. In some cases, lactate may be initially normal, but lactate/pyruvate ratio may be elevated, thus providing an earlier marker of mitochondrial damage[30]. It is established that in inherited mitochondrial diseases, exercise may help in diagnosing early mitochondrial dysfunction, by enhancing the sensitivity of lactate/pyruvate ratio. Therefore, this ratio should be determined at rest and then after exercise in cases where the lactate is normal in the setting of a clinical suspicion of mitochondrial toxicity. Recently, significant decrease in blood mtDNA was found in a small group of subjects with symptomatic hyperlactatemia[40]. Our group found no mtDNA depletion in the blood of two subjects with hyperlactatemia, including one with fulminant lactic acidosis[41]. Therefore, more data is needed on the reproducibility of this assay and its sensitivity in diagnosing early mitochondrial toxicity.

Management of lactic acidosis / symptomatic hyperlactatemia

Subjects with symptomatic hyperlactatemia should have their therapy modified or even temporarily interrupted. In fact, because of the concern for progression to lactic acidosis, most experts would recommend a temporary interruption of all antiretrovirals until patients are asymptomatic and lactate returns to a normal level. This may take several weeks to months[25]. Management of

such patients after the resolution of the serious hyperlactatemia is controversial. Switching to NRTI-sparing regimens would likely represent the safest approach. Other successful approaches have been to switch d4T to less mitochondrial-damaging NRTI, such as abacavir or zidovudine[25,30,38]. Switching d4T to tenofovir is currently under investigation as another alternative for heavily NRTI-experienced individuals. Several trials are underway to investigate the role of antioxidants, B vitamins and carnitine in this setting. Thiamine (vitamin B1) and riboflavin (vitamin B2) have been successfully used in the treatment of lactic acidosis[42-45], as well as in its secondary prevention[46]. Doses used mirrored the ones used for inherited mitochondrial diseases: 50 mg of riboflavin and 100 mg of thiamine. Carnitine is necessary for transporting long-chain fatty acids across the inner mitochondrial membrane for the process of beta-oxidation, a process that occurs mainly in skeletal muscle, heart, and liver. Treatment with L-carnitine has also been considered in inherited mitochondrial diseases because secondary carnitine deficiency frequently exists in this setting. The frequency of carnitine deficiency in HIV infection remains unclear. The typical dose of levocarnitine used for inherited mitochondrial diseases is 100 mg/kg/day for children and 2 to 4 grams per day for adults in three divided doses[47]. Anecdotal reports exist of the use of L-carnitine for treatment of lactic acidosis in HIV-infected patients[43,48].

Asymptomatic hyperlactatemia

Measurement of venous lactate levels is currently used as a non-invasive marker of mitochondrial toxicity. Unfortunately, lactate levels are non-specific and insensitive for the detection of early mitochondrial dysfunction[49]. In fact, the significance of lactate levels in the HIV-infected population is questionable. Several studies found a prevalence of hyperlactatemia of up to 36% of largely asymptomatic NRTI-treated subjects[26,27,39,50,51]. When strict criteria for collection and processing were adopted, only 4% of heavily NRTI-treated HIV-infected subjects had an elevated lactate[52]. This significant variability in the reported rates likely represents a high rate of falsely elevated lactate levels in the former studies. In fact, longitudinal studies have yet to show any prognostic significance of asymptomatic hyperlactatemia[27,53]. Therefore, until well-performed longitudinal studies explore the natural history of lactate elevations, screening asymptomatic NRTI-treated subjects could not be recommended.

Additional non-invasive screening methods for mitochondrial toxicity are urgently needed in HIV-infected subjects. Phosphorus spectroscopy has been extensively used in inherited mitochondrial diseases as a non-invasive marker of mitochondrial function, and is currently under investigation in the HIV population. And, as mentioned above, blood testing for mtDNA depletion is promising, non-invasive and easily obtainable, but its usefulness needs to be demonstrated in larger studies before any recommendation for its routine use could be issued. There should be a low threshold for prompt testing of lactate levels in subjects who experience symptoms consistent with mitochondrial toxicity, in the absence of other plausible etiologies. In these cases, lactate should be drawn without the use of either a tourniquet, or fist clenching, and strictly using a chilled sodium fluoride-potassium (gray-top) tube, with prompt transport and processing of the specimen. Figure 3 details the recommendations of the AIDS Clinical Trials Group Mitochondrial Focus Group on the proper collection and processing of venous lactate levels.

Is lipodystrophy related to NRTI-induced mitochondrial toxicity?

The prevalence of lipodystrophy has been estimated at 7-84%[54-56] among adult patients and 1% to 43% among pediatric HIV-infected patients[57]. Lipodystrophy involves the key component of peripheral fat wasting or lipoatrophy,

1. Have subject sit, relaxed for 5 min prior to venipuncture.
2. Instruct subject to not clench the fist before or during the procedure and to relax the hand as much as possible.
3. If possible, do not use a tourniquet. If a tourniquet is necessary, then apply tourniquet lightly and draw lactate first before the other samples with the tourniquet still in place.
4. Collect the blood in a chilled gray-top (sodium fluoride-potassium oxalate) tube.
5. Place the specimen immediately on ice and send to the laboratory for immediate processing, preferably within 30 min of collection.
6. If random lactate is elevated, then repeat as above with the following additional patient instructions: no alcohol within 24 h, no exercise within 8 h, and no food or drink except water within 4 h of the draw.

Venous lactate levels are highly dependent on collection techniques. It is therefore recommended that the above instructions be closely followed. If carefully collected, venous lactate level is equivalent to an arterial collection in most clinical situations.

Figure 3. *AACTG guidelines for lactate level specimen collection.*

with or without fat accumulation in the abdomen, breast, and/or neck. This fat maldistribution is commonly associated with metabolic abnormalities – dyslipidemias and/or insulin resistance. Initial studies linked lipodystrophy to the use of protease inhibitor (PI) agents[58,59], but more recent observations called into question this association, as significant proportions of PI-*naïve* individuals had developed this syndrome[60-62]. In addition to distressing cosmetic consequences, lipodystrophy has been associated with decreased adherence to antiretroviral therapy[63], hypertension[64], as well as decreased quality of life, self-esteem and sexual difficulties[65].

Recent reports highlight the fact that lipodystrophy, mainly the lipoatrophy component, is primarily linked to NRTI therapy, while dyslipidemias and insulin resistance are more readily associated with PI therapy. A short course of PIs given to HIV-uninfected healthy subjects was able to cause significant dyslipidemias and insulin resistance[66,67]. Recent data indicates that lipodystrophy is very uncommon in subjects who are treated with NRTI-sparing regimens[68,69]. Cohen, et al. reported a minimal prevalence of lipoatrophy in subjects treated exclusively with ritonavir/saquinavir, compared to subjects treated with NRTI in addition to the same duration of ritonavir/saquinavir[68]. These studies suggest that while a small number of NRTI-*naïve* subjects may develop lipoatrophy, this syndrome is much more common when NRTI are added to the PI therapy. They also suggest that PI are not the most important factor in the development of lipodystrophy. In fact, several observational studies have revealed a significant rate of lipoatrophy in NRTI-treated PI-*naïve* subjects[60-62,70].

The recent association of NRTI-associated hyperlactatemia and lipoatrophy led to the attractive hypothesis that mitochondria may be playing a key role in these body fat changes, possibly through the release of apoptosis mediators that in turn lead to peripheral fat loss[71-73]. In an effort to validate this hypothesis, several investigators have examined fat biopsies in patients with lipodystrophy. Significant ultrastructural abnormalities in the adipocytes of these subjects were found, in particular disturbed architecture of the cristae, inclusion bodies, and increased size and number of mitochondria[74,75]. Such ultrastructural changes are very similar to the ones described in the muscle and liver of subjects with NRTI-induced lactic acidosis or symptomatic hyperlactatemia[25,29,37,76]. Three groups separately showed a significant decrease of mtDNA content in the fat of subjects with lipodystrophy[75,77,78]. These results, although supportive of the mitochondrial hypothesis, raised some skepticism about the mitochondrial hypothesis, since this level of mtDNA decrease seen (mean decrease of 44%) may not be sufficient to cause mitochondrial dysfunction in tissues[79]. In addition, there was some significant overlap between mtDNA levels in subjects with lipodystrophy and HIV-uninfected or HIV-infected antiretroviral-*naïve* controls. For example, in one study, 13% of lipodystrophic subjects had normal fat mtDNA levels, while 15% of fat samples from HIV-uninfected controls showed decreased levels[78]. In addition, both respiratory chain dysfunction[16,38,80] and mtDNA deletions[16,80] have been reported in the muscle and fat of subjects with lipodystrophy. In contrary to what has been demonstrated for fat mtDNA, there appears to be no depletion in blood mtDNA of subjects with lipodystrophy[41,78,81]. Only one of these investigations performed a complete analysis of the mitochondrial genome, which revealed several polymorphisms, resulting in amino acid substitutions[41]. This may constitute a possible explanation as to why some NRTI-treated individuals appear to be at a particular risk of developing mitochondrial toxicity.

Further support of the role of NRTI in lipodystrophy comes from the numerous switch studies in which the substitution of NNRTI or abacavir to the PI of a virologically successful regimen did not lead to any improvement in the fat abnormalities, despite significant improvement of dyslipidemias and insulin resistance[82]. On the contrary, recent experience from our group[38] and others[83-85] indicates that changing NRTI, from d4T to either abacavir or zidovudine, does lead to a partial resolution of hyperlactatemia and lipoatrophy, implying a differential action of NRTI. This does support the *in vitro* observations that d4T is a more potent inhibitor of mtDNA than other currently used NRTI[5-9], and the observational studies which revealed a higher rate of lipodystrophy in subjects treated with d4T- vs other NRTI-containing regimen[61,62]. Ongoing studies are exploring the outcome of substituting d4T with tenofovir in patients with lipoatrophy.

Does HIV itself have any effect on mitochondria?

Several observations suggest a possible link between HIV itself and mitochondrial dysfunction. Histological evidence of mitochondrial abnormalities, including red-ragged fibers and abnormal mitochondria with paracrystalline inclusions have been seen in antiretroviral-*naïve* HIV-infected subjects with clinical myopathy[86,87]. A recent study reported a significant decrease in mtDNA, as assessed by mtDNA/nDNA ratio in blood of HIV-infected antiretroviral-*naïve* subjects compared to HIV-uninfected controls[40]. In our cohort, 8% of antiretroviral-*naïve* subjects had elevated blood lactate, compared to only 4% of NRTI-treated subjects[52]. Others showed a similar prevalence of hyperlactatemia in therapy-*naïve* subjects[51]. In addition to these clinical observations, *in vitro* data describe the effect of VPr on mitochondria[88].

Mitochondrial toxicity in HIV-infected and HIV-exposed children

There are several concerning reports about HIV-exposed but uninfected infants who developed hyperlactatemia after perinatal exposure to NRTI therapy. One prospective study examined serial lactate levels in 25 infants born to HAART treated mothers, and reported that 92% of infants had elevated lactate, with 35% of the cohort demonstrating levels >5 mmol/l[89]. Only one infant was symptomatic with irritability and feeding intolerance. Lactate levels were normal in the few mothers who were tested prior to delivery. The hyperlactatemia resolved in all of the infants by six months of age[89]. Another similar study of lactate levels in a group of 20 NRTI-exposed neonates also showed elevated lactate levels in 85% of these infants, none of whom was symptomatic[90]. Similar to the first study, lactate levels returned to normal after the first few weeks of life, without apparent consequence on the infants. Severe mitochondrial dysfunction rarely occurs, with the central nervous system seemingly particularly vulnerable. Blanche, et al. reported eight cases of hyperlactatemia with neurological and developmental sequelae in perinatally NRTI exposed, HIV-negative children[91]. Foster, et al. reported three full-term infants who were exposed to NRTI perinatally, and who experienced lactic acidosis and hypoglycemia[92]. All three infants recovered. Another unique aspect of lactic acidemia in the pediatric population is its likely effect on growth. Chronic acidemia is well known to inhibit linear growth in children and adolescents. It is important to recognize the severe technical difficulties associated with obtaining venous lactate levels in children; the use of a tourniquet and fist clenching are unavoidable in the pediatric population, and may lead to false-elevations of lactate levels. Therefore, the studies showing asymptomatic transient elevations of venous lactate in newborns should be considered with caution[89,90]. Despite the frightening reports of rare mitochondrial dysfunction in NRTI-exposed infants, this risk is still outweighed by the remarkable success in preventing vertical transmission of HIV. In HIV-infected NRTI-treated children, Giaquinto reported transient elevated venous lactate levels in 8% of his cohort, all of whom were asymptomatic[90]. Longitudinal studies are necessary to determine the prevalence and long-term effects of chronic hyperlactatemia in children.

Conclusions

NRTI remain the cornerstone of antiretroviral therapy. It is unclear why some HIV-infected patients have tolerated these medications for more than a decade without any noticeable side effects, while others are experiencing distressing and serious toxicities. The future of antiretroviral development should focus on new drugs with more acceptable short- and long-term toxicity profile. For now, we should focus on early diagnosis and management of the known toxicities, so we may improve the quality of life and long term outcome of HIV-infected subjects.

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