Nucleoside Analogues and Mitochondrial Toxicity

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ABSTRACT

The availability of durable, effective antiretroviral therapy for HIV-infected patients has fundamentally altered the prognosis of this disease and has also increased awareness that long-term drug toxicities have the potential to cause significant morbidity and even mortality in this patient population. Nucleoside analogue reverse transcriptase inhibitors (NRTIs) represent key components of the antiretroviral combinations used to manage HIV infection. Many of the important and treatment limiting side-effects of nucleoside analogues have been suggested to be related to the impact of these agents on mitochondrial DNA polymerase gamma. The long-term use of nucleoside analogue reverse transcriptase inhibitor (NRTI) drugs has been associated with a number of clinically relevant toxicities including hyperlactataemia and lactic acidosis, neuropathy, pancreatitis and lipoatrophy. At present there is no reliable method of detecting subclinical mitochondrial toxicity in patients exposed to NRTIs. Clinical awareness of this problem is therefore important to ensure the early detection of significant side effects and to allow timely consideration of changing therapy in those affected. There is no proven, effective therapy for NRTI-associated mitochondrial toxicity other than ceasing the implicated agent, and even with this strategy, resolution of symptoms may be incomplete. (J Infect Dis Antimicrob Agents 2006;23:27-45.)

INTRODUCTION

The recent availability of highly active antiretroviral therapy (HAART) has dramatically reduced the human immunodeficiency virus (HIV)-associated morbidity and mortality, and substantially improved the long term prognosis of patients with HIV infection. However, the significant increase in life expectancy and the need for permanent antiretroviral treatment have led to the observation of new, frequent, and sometimes severe adverse events associated with the antiretroviral agents. Among the clinically available antiretroviral drugs, nucleoside analogue reverse transcriptase inhibitors (NRTIs) were the first to demonstrate clinical efficacy against HIV infection. The ongoing use of NRTI therapy into the second decade of HIV therapy highlights the need for a comprehensive understanding of NRTI-induced drug toxicities1-3, as HIV infection becomes a manageable disease with greatly reduced...
morbidity and mortality attributable to immune deficiency. This review is intended to provide understanding of the function of mitochondria and to discuss the main clinical toxicities thought to be associated with mitochondrial dysfunction and how they may be managed.

Mitochondria: roles and regulation

The main function of mitochondria is to produce energy for the cell in the form of adenosine triphosphate (ATP), via the process of oxidative phosphorylation. Other biochemical and biological contributions of mitochondria to eukaryotic cellular function have been summarized in Table 1.

Limitations of HIV mitochondrial toxicity research

Mitochondrial (mt) toxicity is a complex phenomenon, with multiple factors involved. Several factors may have contributed to the inconsistencies currently found in the literature on HIV-related mt toxicity (Table 2). Firstly, the relatively low incidence of severe mt toxicity-related adverse events has presented a challenge to studies in the HIV patient population. Secondly, many clinical symptoms suspected to have their etiology in mt toxicity such as peripheral neuropathy, myopathy or even lipoatrophy can be challenging to quantify objectively in a standardized fashion. In certain tissues with rapid turnover such as blood cells, depleted mtDNA levels usually ‘rebound’ upon withdrawal of drug pressure. This readily available sample is thereby susceptible to treatment interruptions, planned or not. Finally, differences in the type of sample studied as well as the techniques and methodologies used could also explain some of the discrepancies in the literature.

Table 1. Main roles and characteristics of mitochondria.

- ATP production through the oxidative phosphorylation
- Synthesis of components of most proteins of the respiratory chain
- Involvement in the control of intracellular Ca²⁺ homeostasis (in matrix granules)
- Generation of, and defense against reactive oxygen species
- Connections to signaling partners such as the endoplasmic reticulum or the plasma membrane for maintenance of electrochemical gradients, and formation of clusters of organelles as long as 50 µm to form intracellular power-transmitting protonic cables
- Regulation of apoptosis by storage and release of molecules that induce apoptosis (cytochrome c and apoptosis-inducing factor), or by inhibitory molecules with anti-oxidant properties
- Import of metabolites across the outer mitochondrial membrane by the pore-forming voltage-dependent anion channel, and storage of least 1,500 proteins encoded by the nuclear DNA, that are translocated into the mitochondria
- Synthesis of heme group in hepatocytes and blood cell precursors
- Intracellular location for peripheral benzodiazepine receptors
- Involvement in the initial step of steroidogenesis, as well as in the intracellular homeostasis of sex steroid hormones
- Production of heat (in brown-fat tissue) through the action of the protein thermogenin (UCP-1) that uncouples oxidation and phosphorylation
- Involvement in cellular ageing with loss or damage of mitochondrial DNA
- Involvement in genetic pathologies due to mutations of mitochondrial DNA
Potential pathways of mitochondrial toxicity

mtDNA depletion (Figure 1)

It has long been known that NRTIs used in HIV therapy can inhibit the mt polymerase γ enzyme in vitro. 

Cell culture and animal studies provide strong evidence in support of the NRTI inhibiting mtDNA synthesis model. mtDNA depletion was first reported in muscle, fat and nerve tissue of HIV patients.

Table 2. Limitations and factors that may contribute to the inconsistency of findings in the HIV antiretroviral therapy mt toxicity literature.

<table>
<thead>
<tr>
<th>Limitations/Factors</th>
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<tr>
<td>Low incidence of severe adverse event</td>
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<tr>
<td>Challenge to objectively grade or quantify certain adverse effects</td>
</tr>
<tr>
<td>Time lag between mt damage and clinical symptoms</td>
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<tr>
<td>Rapid cell turnover</td>
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<tr>
<td>Limited sample size</td>
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<tr>
<td>Cross-sectional as opposed to longitudinal design</td>
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<tr>
<td>Potential biases inherent to observational studies</td>
</tr>
<tr>
<td>Differences in sample type and sample processing</td>
</tr>
<tr>
<td>Differences in techniques and methodologies</td>
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</tbody>
</table>

1. Direct mtDNA depletion through inhibition of replication by polymerase γ and early chain termination
2. Direct mtRNAs (mRNA, tRNA or rRNA) depletion through inhibition of RNA polymerase or down-regulation of transcription
3. Indirect depletion of mtDNA and mtRNAs through nucleotide pool imbalance caused by:
   3.1. MRC dysfunction inhibiting de novo pyrimidine synthesis
   3.2. Reduced ATP production inhibiting endogenous nucleoside phosphorylation
   3.3. NRTI phosphorylation by mt kinases inhibiting endogenous nucleoside phosphorylation
   3.4. Direct inhibition of MRC by non-phosphorylated nucleoside analogues

Figure 1. Schematic representation of several potential mechanisms by which NRTIs could affect mtDNA and mt gene expression (mtRNA), leading to mt toxicity [Adapted from Cote HC. Possible ways nucleoside analogues can affect mitochondrial DNA content and gene expression during HIV therapy. Antivir Ther 2005;10 Suppl 2:M3-11.]
experiencing drug-related symptoms of myopathy, lipoatrophy and neuropathy, respectively. However, whether this mtDNA depletion is the pathogenesis for all clinical symptoms of toxicity observed in HIV patients on HAART is less clear. The DNA polymerase $\gamma$ hypothesis by itself fails to explain the entire array of metabolic deficiencies associated with NRTI-induced disorders. The mtDNA depletion was reversible upon removal of the NRTIs suspected of causing mt toxicity, as are most toxicity symptoms if the antiretroviral therapy is changed or interrupted but only after at least 6 months of interruption. The combination of stavudine (d4T) with didanosine (ddI) was also associated with a greater risk of hyperlactataemia, and greater blood mtDNA depletion than the other NRTI combinations studied. Discrepancy and controversy also remains with respect to the effect of NRTI-mediated mtDNA depletion on mt function. Several studies, using various tissues from HIV patients, have concluded that mtDNA levels and mt activities are positively correlated. However, others would suggest otherwise and the relationship between mtDNA and mt function is somewhat unclear based on the current HIV literature. For example, one study found that a decrease in blood cell mt mass and mtDNA content was not accompanied by a decrease in cytochrome C oxidase gene expression or activity, leading the authors to suggest a compensatory mechanism up-regulating mt transcription or translation. Another study from the same group found that decreased peripheral blood mononuclear cell (PBMC) mtDNA was accompanied by a decrease in mt respiratory chain (MRC) complex IV activity, but without evidence of mt dysfunction such as altered oxygen consumption or lipid peroxidation. Indeed, results have also been inconsistent with respect to the association of mtDNA depletion with specific clinical adverse effects widely attributed to mt toxicity such as lipoatrophy and hyperlactataemia. For example, in cases of hyperlactataemia and lipoatrophy, several studies have shown an association between the symptoms and mtDNA depletion in blood cells, while others have not. Overall, poor correlation between mtDNA levels and various mt toxicity symptoms strongly suggest additional mechanism(s), some of which are presented below.

Mitochondrial RNA depletion

Interestingly, the DNA polymerase $\gamma$ is capable of catalyzing reverse transcription with a high efficiency, an activity that may be physiologically significant, especially considering that RNA-primed DNA synthesis activity is required for initiation of mtDNA replication. Such activity could also be inhibited by NRTIs. Another potential target of NRTIs could be the mtRNA polymerase itself and the co-factors required for mt transcription, some of which are regulated through phosphorylation and are, therefore, susceptible to MRC dysfunction. In addition, NRTIs could impair ribonucleotide synthesis and/or utilization in a manner similar to that hypothesized for nucleotides. All of the above could potentially translate into altered mt messenger RNA (mRNA) levels.

Nucleotide pool imbalance

Imbalances in the mt nucleotide pool have been suggested as the cause of mtDNA abnormalities in patients with inherited thymidine phosphorylase deficiencies. In a similar manner, nucleoside analogues used to treat HIV infection can alter the endogenous cellular and mt nucleoside/nucleotide pools, potentially leading to the disturbance of a wide range of nucleic acid pathways that depend on these building blocks (Figure 1).

Mitochondrial DNA damage (mutation, deletion)

Much attention has been paid to mtDNA quantity
in recent research. Also important, but technically challenging to evaluate, is mtDNA quality. It is well-documented that mtDNA damages through the accumulation of mutations and deletions over time, and plays a role in the conditions and diseases associated with aging.\(^{43,44}\) NRTI therapy also provides conditions permissive for the development of peripheral blood mtDNA mutations in vivo.\(^{45}\) Furthermore, NRTI or oxidative agents have been shown to directly impair the energy-producing system of mitochondria, causing dysfunction of cellular redox control, which eventually leads to loss of the mtDNA integrity.\(^{46}\) The clinical importance of this effect has not been established, although it appears to be a relatively rare cause of disease. Nevertheless, neurological syndromes attributable to mtDNA mutations have been described in association with NRTI therapy, including five published cases of Leber’s hereditary optic neuropathy.\(^{47-50}\)

**NRTIs and mitochondrial DNA**

After HIV has entered the cell, it is required to integrate with the host cell genome. To do this, it needs to convert single stranded viral RNA into double stranded DNA and this task is performed by the enzyme reverse transcriptase. The NRTIs resemble the natural nucleosides but do not have a free 3’ hydroxyl group, and thus once they are added to the growing DNA chain, termination occurs. Mitochondria are the energy powerhouses of cell and are unique among intracellular organelles in containing their own genome that is extrachromosomal - replicating independently of nuclear DNA. Hence, mtDNA can be synthesized in post-mitotic and resting cells, utilizing a unique DNA polymerase gamma whose evolutionary origins\(^{51}\) and enzymatic activities\(^{52}\) are distinct from the multiple DNA polymerases involved in nuclear DNA synthesis. Importantly, DNA polymerase-gamma is also unique among the human polymerase in that it is unable to discriminate between naturally occurring nucleotides and nucleosides analogue drugs.\(^{51}\) Hence, NRTIs are capable of inhibiting cellular mtDNA synthesis via their effects on DNA polymerase-gamma (Figure 2). The proposal that NRTI-induced polymerase-gamma inhibition represents a common pathway for tissue-specific adverse effects of this drug class has been enunciated by Lewis & Dakalas\(^{53}\) and Brinkman et al\(^{54}\), and reviewed more recently by Nolan\(^{55}\) and Cote.\(^{10}\) The recognition that NRTIs may interfere with mitochondrial DNA synthesis led to many studies evaluating these effects in vitro, extensively reviewed by Kakuda.\(^{1}\) Studies in neuronal cell models showed that the ddC, ddI, and d4T caused toxicity, whereas AZT and 3TC did not, again reflecting what was seen in clinical practice.\(^{56}\) It is worth stating at the outset that the toxic and therapeutic profiles of individual NRTI drugs are not correlated, so that an increased efficacy is not necessarily accompanied by increased toxicity.\(^{57}\) These studies suggested a ranking of zalcitabine (ddC) > didanosine (ddI) > stavudine (d4T) > lamivudine (3TC) > zidovudine (AZT) > abacavir (ABC) for effects on mitochondrial polymerase gamma. Another important factor to consider is the anabolism of the NRTIs, since these agents need to be phosphorylated three times before they can be added to the growing DNA chain by HIV reverse transcriptase or other polymerases (Figure 2). This process is known to vary with the activation state of the cell, with d4T and AZT being more active in activated cells and other NRTIs being more active in resting cells. The entry of NRTIs into cells has been observed to occur at different rates, and there are many transport systems available for nucleosides. Since the phosphorylation of NRTIs may differ between subcellular compartments, it follows that movement of the drugs and their anabolites between the cytosolic and mitochondrial compartments is of considerable interest.\(^{58}\) Early studies with 3TC showed...
synergistic or additive activity against HIV in vitro, and also protection against the delayed mitochondrial toxicity associated with d4T, AZT, ddC, and ddI.59 The protection conferred by 3TC in this study was thought to be due to interference with the uptake of the other agents into mitochondria. Further understanding of the activation and transport of the NRTIs within different subcellular compartments may lead to molecules or strategies in which efficacy can be enhanced and toxicity reduced.

HIV and mitochondria

It is known that many of the toxicities associated with NRTI therapy may also be related to HIV infection itself, but it is not often appreciated that there is also known to be a direct interaction between HIV and mitochondria. Early studies showed that HIV RNA could be found in mitochondria of infected cells, and that there were mitochondrial alterations in patients with the acute HIV syndrome and stable infection. More recent work has shown that the HIV TAT protein may promote mitochondrially induced apoptosis60, consistent with our knowledge of the importance of this process in the immune cell destruction caused by the virus. A specific interaction between the HIV viral protein R and the mitochondrial permeability transition pore complex (PTPC) has recently been demonstrated by Jacotot and colleagues61, and with the recognized involvement of the PTPC in apoptosis it seems likely that HIV affects the immune system at least in part by

Figure 2. The basis of NRTI-associated mitochondrial toxicity according to the ‘pol-gamma’ model NRTI drugs differ from natural nucleoside compounds in that the 5’-hydroxyl group (indicated by arrow) is modified or removed, so that incorporation into a nascent DNA chain leads to chain termination. Note that the affinity of a given NRTI drug for DNA polymerase-gamma (a determinant of its ability to cause mtDNA depletion) is not related to its affinity for HIV reverse transcriptase (a determinant of antiretroviral efficacy). NRTI-MP, NRTI-monophosphate; NRTI-DP, NRTI-diphosphate; NRTI-TP, NRTI-triphosphate. [Adapted from Nolan D, Mallal S. Complications associated with NRTI therapy: update on clinical features and possible pathogenic mechanisms. Antivir Ther 2004;9:849-63.]55
interacting with mitochondria leading to programmed cell death. Such viral effects are not uncommon; indeed the hepatitis B virus protein X has also been shown to interact with a component of the PTPC.

Clinical toxicity of the NRTIs

NRTIs are associated with a wide spectrum of toxicities, many also caused or exacerbated by HIV itself. While the prototypic NRTI-associated toxicity syndrome is zidovudine myopathy, this syndrome is now rare in clinical practice, possibly reflecting either the use of lower AZT doses in the HAART era or the involvement of uncontrolled HIV infection per se in the pathogenesis of this syndrome. Currently, the adverse effects of most concern involving NRTI therapy are lactic acidosis and other less serious disorders of lactate metabolism, the progressive subcutaneous fat wasting syndrome that is viewed as part of the lipodystrophy syndrome, neuropathy and pancreatitis. The major toxicities associated with NRTI therapy will now be reviewed (Table 3). Proposed clinical manifestations of mitochondrial toxicity associated with different NRTIs are listed in Table 4.

Lactic acidosis and hyperlactemia

Lactic acidosis with or without hepatic microsteatosis is the most serious presentation of NRTI-associated

Table 3. Clinical syndromes attributed to NRTI-associated mitochondrial toxicity.65

<table>
<thead>
<tr>
<th>Non-tissue-specific mitochondrial toxicity syndromes</th>
<th>(i) Tissue-specific mitochondrial toxicity syndromes</th>
</tr>
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<tbody>
<tr>
<td><strong>Lactic acidosis</strong></td>
<td><strong>Myopathy</strong></td>
</tr>
<tr>
<td>associated with NRTIs</td>
<td>Associated with AZT</td>
</tr>
<tr>
<td>abdominal pain, lethargy, tachypnea, neuromuscular weakness</td>
<td>Myalgia, weakness</td>
</tr>
<tr>
<td>may be asymptomatic</td>
<td>Very rare in clinical practice</td>
</tr>
<tr>
<td>often un heralded, acute onset</td>
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<tr>
<td>elevated lactate, decrease HCO$_3^-$</td>
<td></td>
</tr>
<tr>
<td>~ 0.1 case/100 patient-years</td>
<td></td>
</tr>
<tr>
<td>high mortality: ~ 30%</td>
<td></td>
</tr>
<tr>
<td><strong>Infantile NRTI toxicity syndrome:</strong></td>
<td><strong>Lipoatrophy</strong></td>
</tr>
<tr>
<td>developmental delay, seizures</td>
<td>Associated with d4T &gt; AZT</td>
</tr>
<tr>
<td>MRI abnormalities</td>
<td>Fat loss: especially legs, face &gt; trunk</td>
</tr>
<tr>
<td>Hyperlactemia</td>
<td>Measurable with DEXA scans - subclinical changes common</td>
</tr>
<tr>
<td>Rare: &lt;0.3% 18-month incidence</td>
<td>No biochemical features</td>
</tr>
<tr>
<td>Prognosis uncertain</td>
<td>Common: d4T ~ 40%, AZT ~ 15% at 30 months</td>
</tr>
<tr>
<td><strong>Adult ‘mitochondrial disease’ syndromes:</strong></td>
<td><strong>Neuropathy</strong></td>
</tr>
<tr>
<td>Leber neuropathy described</td>
<td>Associated with ddC &gt; ddI,d4T</td>
</tr>
<tr>
<td>Bilateral optic neuritis</td>
<td>Painful sensory neuropathy</td>
</tr>
<tr>
<td>Likely in genetically susceptible individuals, may have positive family history</td>
<td>Abnormal electrophysiology - subclinical changes common</td>
</tr>
<tr>
<td></td>
<td>No biochemical features</td>
</tr>
<tr>
<td></td>
<td>Common: ddC/ddI/d4T 40%</td>
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</table>
mitochondrial dysfunction. It was first identified in the era of NRTI monotherapy but cases have continued to be reported with dual and triple combination therapy. Calza and colleagues have recently reviewed this area and other reviews have been published previously consequently this section will focus on recent data. Lactic acidosis has been reported in persons receiving both single- and dual-NRTI regimens including combinations of AZT or d4T with ddI, ddC or 3TC.

**Classification**

Hyperlactatemia is defined as mild to moderate increase in serum lactate concentration (2-5 mmol/L), with normal pH value and bicarbonate level (pH > 7.35 and bicarbonate concentration > 20 mmol/L). At one end of this spectrum is a relatively common syndrome of mild, asymptomatic, non-progressive hyperlactatemia, which appears to represent a ‘compensated’ homeostatic system in which elevated lactate clearance. This usually occurs in situations where tissues are well-perfused and buffering systems are able to prevent a fall in pH and metabolic acidosis. While the degree of hyperlactatemia appears to be greater in the presence of d4T or ddI therapy compared with AZT or ABC, this syndrome appears to be benign, irrespective of the choice of NRTI therapy and unlikely to progress to lactic acidosis. In contrast, lactic acidosis and hepatic steatosis represents a relatively uncommon but life threatening clinical syndrome in which lactate homeostasis is completely ‘decompensated’, allowing the rapid, progressive accumulation of lactate and development of acidosis in affected patients.

Lactic acidosis is defined as persistently and remarkably elevated serum lactate level (generally > 5 mmol/L), associated with metabolic acidosis (pH < 7.35 and bicarbonate concentration < 20 mmol/L). Healthy individuals have circulating levels of lactate but maintain normal blood pH. Lactate levels may rise during periods of increased energy needs. However, hyperlactatemia does not inevitably lead to acidosis. Recent studies has shown that asymptomatic hyperlactatemia is relatively common in patients receiving NRTIs (~ 15-35%). On the contrary, the incidence of symptomatic hyperlactatemia and lactic acidosis varies from 1.2 to 25.2 cases per 1,000 person-years of treatment with NRTIs, with a high variation in the calculated frequency due to the variety of case definitions employed. Severe lactic academia has been described in association with all NRTIs, particularly if treatment duration is > 6 months.

An ‘intermediate’ syndrome or symptomatic hyperlactataemia has also been described characterized by symptomatic hyperlactatemia or hepatic steatosis without systemic acidosis. This syndrome is almost uniformly associated with d4T therapy, with an incidence

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**Table 4. Proposed clinical manifestation of mitochondrial toxicity.**

- Hyperlactataemia/lactic acidosis: d4T > ddI > ZDV > other
- Lipoatrophy: d4T > ZDV > other NRTIs
- Peripheral neuropathy: ddC > d4T > ddI
- HIV-associated neuromuscular weakness syndrome: d4T > other NRTIs
- Pancreatitis: ddI > d4T
- Hepatic steatosis: d4T > other NRTIs
- Skeletal myopathy/ cardiomyopathy: ZDV
- Adverse effects on maternal/fetal health: d4T + ddI
of approximately 13 per 1,000 person-years. Of importance, there is evidence that lactate levels and symptoms can be controlled following modification of NRTI therapy to AZT or ABC.

**Risks for hyperlactatemia** (Table 5)

The risk of lactic acidosis may vary with demographics, being somewhat more common in woman, the obese and those with concurrent liver disease and by nucleoside combination, several cohorts suggesting d4T + ddI being associated with the greatest relative risk.

The median duration of antiretroviral therapy before the onset of symptoms was 9 months (range 3-20). The most common complaints are fatigue, weight loss, myalgias, nausea and vomiting, abdominal distension, pain, and dyspnea.

**Management**

In symptom-free patients, only serum lactate concentrations of or above 5.0 mmol/L warrant discontinuation of therapy. However, thresholds as high as 10 mmol/L have been suggested by other authors. The management of mild (ie, 2-5 mmol/L), asymptomatic hyperlactatemia is less certain. As a first step, the test should be repeated and artificial causes excluded (Figure 3). Artificial causes include blood samples drawn too close to periods of exertion, improper use of tourniquets, failure to use fluoride-oxalate containing tubes, failure to preserve samples on ice during transport, and significant delays in laboratory analysis. However, in the absence of these confounding variables, current recommendations permit cautious continuation of NRTIs while monitoring the patient closely for the development of symptoms and/or further increases in lactate (Figure 3).

Once all other causes have been excluded, prompt withdrawal of all NRTIs is the cornerstone of management (Figure 3). Despite the absence of controlled data, most authorities also recommend the administration of intravenous fluids to pre-empt cardiovascular collapse and assist hepatic and renal clearance of lactate. More controversial, however, is the use of intravenous sodium bicarbonate, which may trigger or compound respiratory acidosis in cases complicated by encephalopathy or coma. The use of so-called ‘specific treatments’ for lactic acidosis - i.e., riboflavin, thiamine, carnitine, co-enzyme Q, and vitamin C - all of which are either cofactors in oxidative phosphorylation or antioxidants, has been bolstered by an uncontrolled, retrospective study that reported exposure to any of these agents correlates with survival. However, dosages and dosing schedules

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**Table 5. Risks for developing hyperlactatemia.**

<table>
<thead>
<tr>
<th>Risk Factor</th>
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<tr>
<td>NRTI (d4T, ddI, ddC, AZT, 3TC)</td>
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<tr>
<td>Long duration of NRTI therapy, particularly if treatment is &gt; 6 months</td>
</tr>
<tr>
<td>Female sex</td>
</tr>
<tr>
<td>Pregnancy</td>
</tr>
<tr>
<td>High BMI</td>
</tr>
<tr>
<td>Pre-existing liver disease</td>
</tr>
<tr>
<td>Viral hepatitis coinfection</td>
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<td>Concomitant use of other hepatotoxic drugs</td>
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have not yet been standardized. The low toxicity potential of these agents, however, makes them an attractive adjunct to standard measures.

Resolution of lactic acidosis after drug discontinuation can be extremely slow; reported experience is from 4 to 28 weeks. Mortality rates overall were high (25-57%). Falco and coworkers reported a serum lactate level > 10 mmol/L as the only factor associated with higher mortality in the multivariate analysis. Anecdotal case reports suggest that resumption of an altered ART regimen is safe after resolution of signs and symptoms. Nucleoside analogues that have little or no mitochondrial toxicity should be utilized (e.g. 3TC, ABC, tenofovir).

Screening

Screening of lactate is of limited use in asymptomatic individuals undergoing antiretroviral therapy. Raised lactate levels represents part of a spectrum of lactate and acid-base disturbance and does not correlate with progression to lactic acidosis. Routine measurement is justified at this time only in NRTI recipients with clinical features consistent with lactic acidosis, such as new-onset fatigue, dyspnea, weight loss or nausea, low bicarbonate, chloride, or albumin, raised anion gap, unexpected increase in liver enzymes, or new onset of clinical liver failure, as well as in pregnant woman receiving NRTIs, and in those recommencing NRTIs who have had lactic acidosis previously.

**Peripheral neuropathy**

In general, the relative risk of toxic polyneuropathy associated with specific NRTI drugs correlates well with ability to inhibit mtDNA polymerase-gamma in vitro. The clinical syndrome common to both HIV-associated and toxic sensory neuropathy is dominated by peripheral pain and dysaesthesia, with rare motor involvement. The greatest risk has been associated with ddC, an NRTI.

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**Figure 3. Algorithm for the management of hyperlactataemia in HIV-infected patients taking NRTIs.**

Asymptomatic plus lactate < 5.0 mmol/L

- Normal lactate
  - End evaluation
- Retest and rule out artifactual causes
  - Hyperlactatemia (> 2 - 2.5 mmol/L)
    - Presence of symptoms or lactate ≥ 5.0 mmol/L
      - Still raised
        - Continue NRTIs cautiously Monitor for symptoms Repeat lactate weekly
      - Stop NRTIs

If persistently 2.1-5.0 mmol/L without symptoms, consider substituting culprit NRTI with abacavir, tenofovir, or lamivudine, or changing to NRTI-sparing HAART regimen.
with equivalent affinity for mtDNA polymerase gamma and HIV reverse transcriptase, thus providing for a narrow therapeutic window.\textsuperscript{32} Neuropathy was a consistent and dose-limiting toxicity in trials of ddC, occurring in approximately one-third of patients receiving low-dose therapy (2.25 mg/d) for less than a year.\textsuperscript{110,111} Non-drug-related factors also found to be associated with an increased risk of peripheral neuropathy, in populations aged 40 and higher.\textsuperscript{32}

Moore et al\textsuperscript{112} analyzed >1,100 patients from the Johns Hopkins AIDS Service to identify risk factors for peripheral neuropathy. The risk of neuropathy was found to be additive and possibly even synergistic for the combination of d4T + ddI + hydroxyurea compared with ddI alone, d4T alone, and d4T + ddI in combination (Table 6).

When a patient presents with peripheral neuropathy, it is important to exclude other possible causes or contributing factors, such as cytomegalovirus infection, syphilis, vitamin B12 deficiency, thyroid dysfunction, alcoholism, diabetes mellitus, and use of other neurotoxic agents.

Treatment

Management of NRTI-associated peripheral neuropathy may involve discontinuation or dose reduction of the responsible agent, although there is a lack of data on the efficacy of this management strategy. Even after the drug is stopped, a substantial proportion of patients may continue to experience chronic pain. The main purpose of treating neuropathy is to relieve symptoms. In mild cases, where symptoms are not affecting daily life, standard pain killers such as ibuprofen and acetaminophen may be all that is necessary.\textsuperscript{113} For more severe symptoms, a number of studies have reported varying success of tricyclic antidepressants such as nortriptyline and amitriptyline, anticonvulsants such as lamotrigine\textsuperscript{114} and gabapentin.\textsuperscript{115,116} More recently, pathophysiologic treatment such as recombinant human nerve growth factor and acetyl-L-carnitine has also been studied with favourable outcome.\textsuperscript{117}

Adipose tissue effects and peripheral lipoatrophy

Clinical studies have demonstrated that NRTI therapy alone provides sufficient conditions for the development of lipoatrophy\textsuperscript{118} and that NRTI therapy is an independent risk factor for its occurrence in HAART-treated individuals.\textsuperscript{119} Several studies reported data pointing toward apoptosis as the main mechanism mediating lipoatrophy.\textsuperscript{120,121} Clinical trials data have now confirmed the findings of observational studies that d4T therapy is associated with an approximately twofold increased risk of lipoatrophy compared with AZT, so that the risk of clinically apparent lipoatrophy over 30 months is approximately 10-20 percent in AZT-treated individuals and 40-50 percent among those treated with stavudine.\textsuperscript{122,123} The role of NRTI drugs other than

<table>
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<tr>
<th>Variable</th>
<th>Hazard ratio</th>
<th>95% CI</th>
<th>P value</th>
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<tbody>
<tr>
<td>ddI</td>
<td>1.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>d4T</td>
<td>1.39</td>
<td>0.84 - 2.32</td>
<td>0.20</td>
</tr>
<tr>
<td>ddI + hydroxyurea</td>
<td>2.35</td>
<td>0.69 - 8.07</td>
<td>0.18</td>
</tr>
<tr>
<td>ddI + d4T</td>
<td>3.50</td>
<td>1.81 - 6.77</td>
<td>0.001</td>
</tr>
<tr>
<td>ddI + d4T + hydroxyurea</td>
<td>7.80</td>
<td>3.92 - 15.5</td>
<td>0.0001</td>
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</table>
d4T and AZT as risk factors for lipoatrophy has been more difficult to discern, particularly as 3TC and ddI are generally used in combination with these thymidine analogues drugs. Thus far, no independent effect of these ‘second’ NRTI drugs has emerged, with results from the available clinical trials completed to 30 months showing similar risk of lipoatrophy if d4T is combined with ddI or with 3TC.\textsuperscript{122,123} Identical features were observed in the presence or absence of protease inhibitor therapy, suggesting that PIs may not affect this parameter. The NRTI-lipodystrophy syndrome differed from protease inhibitor-related lipodystrophy syndrome by the presence of recent onset symptoms and weight loss, higher lactate and alanine aminotransferase, and lower albumin, cholesterol, triglycerides, glucose and insulin.\textsuperscript{74}

In contrast to the lack of improvement associated with discontinuation of PIs, several studies have demonstrated improvement in lipoatrophy with NRTI switches. In MITOX study, lipoatrophic HIV-infected adults, switching from stavudine or zidovudine to abacavir for 24 weeks led to significant, albeit modest, objectively measured increases in limb fat.\textsuperscript{124} However, clinical lipoatrophy, as assessed subjectively, did not resolve even in long-term follow-up.\textsuperscript{125}

**Pancreatitis**

There is a differential diagnosis for pancreatitis occurring in an HIV-infected individual that includes NRTI-associated disease, in which that pancreatitis may be an isolated event or may occur in the context of systemic lactic acidosis.\textsuperscript{126} Other causes must also be considered, including secondary pancreatitis to severe HIV PI-induced hypertriglyceridemia and the effects of other drugs such as pentamidine.

Moore et al again analyzed patients from the Johns Hopkins AIDS Service to identify risk factors for pancreatitis ( n=2,613, 33 cases).\textsuperscript{126} In this analysis, ddI and d4T were associated with roughly equivalent risk of pancreatitis, with estimated incidences rates of 0.8 and 1.1 cases per 100 person-years, respectively. AZT therapy was associated with an incidence of 0.1 per 100 person-years. Combining d4T and ddI increased risk approximately eightfold.

**HIV-associated neuromuscular weakness syndrome (HANWS)**

Another neurologic condition that may be caused by NRTI-associated mitochondrial toxicity is HANWS. This syndrome presents with rapidly progressive severe neuromuscular weakness associated in most cases with hyperlactatemia. It has been described in 69 patients receiving NRTIs, 61 of whom were on d4T-containing regimens.\textsuperscript{127} Neurologic features observed in these patients included ascending paresis in 100 percent, sensory symptom in 32 percent, areflexia in 17 percent, and bulbar symptoms in 12 percent. Outcomes reported for 44 of these patients indicated that 16 recovered, 19 had residual weakness requiring the use of a wheelchair after months, and 9 died, generally as a result of lactic acidosis and multiorgan failure. For many of the patients who died, a causal connection to NRTIs was not recognized and the NRTIs had not been stopped.

**CONCLUSION**

The benefits of HAART are clear. However longer survival and prolongs use of NRTI have led to more obvious long-term complications especially mitochondrial toxicity. Clinical syndromes mediated by mitochondrial toxicity include hyperlactatemia and lactic acidosis, lipoatrophy, peripheral neuropathy, and hepatic steatosis. Close monitoring for these syndrome requires a tissue-specific approach. Early recognition of mild or subclinical disease, especially neuropathy and lipoatrophy, increases the likelihood that these syndromes will be reversible with appropriate
management, which includes discontinuation of the offending agents.

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