Adverse effects of reverse transcriptase inhibitors: mitochondrial toxicity as common pathway

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AIDS 1998, 12:1735–1744

Keywords: HIV infection, nucleoside analogues, non-nucleoside analogues, nucleotide analogues, drug reactions, human DNA polymerases, DNA polymerase γ, mitochondrial toxicity

Introduction

After zidovudine (ZDV), a 3'-azido analogue of thymidine, was found to be an effective antiretroviral drug against HIV [1,2], other nucleoside analogues inhibiting reverse transcriptase (RT) soon followed: didanosine (ddI), zalcitabine (ddC), lamivudine (3TC), stavudine (D4T), and recently abacavir (1592U89) [3–7]. These drugs have demonstrated efficacy in reduction of morbidity and mortality, especially in combination therapy [8–10]. A special feature of some of these drugs is the protection against AIDS dementia complex, which appears to be related to good penetration of the blood–brain barrier [11–13]. Although the introduction of protease inhibitors has changed the management of HIV infection drastically, this cerebroprotective property will assert the role of these nucleoside RT inhibitors (NRTI) as a cornerstone of antiretroviral therapy [9,10].

More than 10 years of experience with NRTI therapy has revealed important adverse effects ranging from mild (myopathy) to fatal in some cases (pancreatitis, liver failure and lactic acidosis). Behind most of these side-effects there appears to be a common mechanism: a decreased mitochondrial energy-generating capacity.

In this review we will summarize the literature in which this mechanism is analysed and will emphasize the importance of acquired mitochondrial dysfunction that will accumulate during long-term treatment with antiretroviral nucleoside analogues.

Nucleoside analogues and DNA polymerases

During the synthesis of DNA, the DNA duplex is unwound by a helicase and each DNA strand directs the synthesis of a complementary DNA strand to generate two DNA duplexes. New nucleotides (triphosphorylated nucleosides: dATP, dCTP, dGTP and dTTP) are added to a pre-existing polynucleotide strand (primer) by an enzymatically catalysed formation of a phosphate ester between the 3'-hydroxyl group of the sugar residue of the nucleotide of the primer and the 5'-phosphate group of the nucleotide to be added. An original DNA strand serves as a template during this process. The enzymes that catalyse this formation of new DNA strands on a template are called DNA polymerases. In eukaryotic cells five types of DNA polymerase are active (DNA polymerase α, β, γ, δ and ε), which all utilize a DNA strand as template. HIV encodes a DNA polymerase (RT) that uses RNA as template. All DNA polymerases have in common the utilization of dNTP as substrate [14–16]. Modification
of dNTP can affect the functioning of DNA polymerases: 2',3'-dideoxy analogues of dNTP (so-called ddNTP) can serve both as inhibitors and substrates of certain DNA polymerases. Since these ddNTP lack the hydroxyl group in the 3'-position, incorporation of a ddNTP will terminate primer elongation (Fig. 1). This mechanism forms the basis of dideoxy sequencing of DNA [17,18] and it was found that ddNTP also inhibited the proliferation of HIV by inhibiting RT [19,20]. All currently used NRTI, such as ZDV, ddC, ddI, 3TC, D4T and abacavir, are dideoxynucleosides, which are phosphorylated intracellularly by host kinases to ddNTP. Since every NRTI (as a ddNTP) might not only inhibit viral RT but also human DNA polymerases, serious toxicity can be expected.

For all currently used NRTI, the interaction with human DNA polymerases has been studied (Table 1): in general, DNA polymerase α, δ and ε are insensitive to inhibition by ddNTP, but both DNA polymerases β and γ can be inhibited in vitro by these compounds [15,21–28]. Fortunately, during the cell cycle DNA polymerase α and δ are responsible for the necessary DNA duplication and NRTI apparently do not interfere in this process. DNA polymerases β and ε are involved in DNA repair mechanisms [14] and so far little is known whether the inhibitory effect of NRTI on DNA polymerase β has any pathophysiological importance. However, since DNA polymerase γ is the only DNA polymerase involved in mitochondrial DNA (mtDNA) replication, the inhibitory action of NRTI on this enzyme can easily interfere in mitochondrial replication and function [16,24,28–30]. Interestingly, as an exception to the other NRTI, 3TC is both an inhibitor of the polymerase activity and a substrate of the integral 3'-5' exonuclease activity of DNA polymerase γ, which makes incorporation less feasible.

Other RT inhibitors (RTI) are also used as HIV inhibitors, including non-nucleoside analogues such as nevirapine, delavirdine, efavirenz [31–33], and nucleotide analogues such as adefovir [9-(2-phospho-methoxyethyl)adenine (PMEA)] [23,34]. Although data on the affinity for human DNA polymerases are less abundant for these compounds, it appears that non-nucleoside analogues do not interfere with any of these polymerases, whereas the currently available nucleotide analogues appear to have a strong affinity for DNA polymerases β and γ in particular [23,34] (Table 1).

### Mitochondrial function and replication

Mitochondria, subcellular organelles present in all cells except erythrocytes, contain the enzymes, enzyme complexes and proteins necessary for the intramitochondrial generation of ATP and its exportation to the cytoplasm.

#### The oxidative phosphorylation system

The most important function of mitochondria is oxidative phosphorylation: the oxidation of fuel molecules by oxygen and the concomitant energy transduction into ATP [35]. The synthesized ATP is used for energy-requiring reactions in the matrix or exported to the cytosol by the adenine nucleotide translocator in exchange for cytosolic ADP [35]. The oxidative phosphorylation system consists of the four multisubunit enzyme complexes of the mitochondrial respiratory chain (complexes I–IV) and the F₁–F₀ ATP synthetase complex (complex V). All are embedded in the lipid bilayer of the inner mitochondrial membrane (Fig. 2). Besides ATP production via the oxidative phosphorylation system in mitochondria, the process of anaerobic glycolysis (i.e., the conversion of glucose to lactate) in the cytoplasm delivers energy. However, glycolysis produces little ATP compared with the oxidative phosphorylation.

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**Table 1.** Kinetic interactions of reverse transcriptase inhibitors with human DNA polymerases.

<table>
<thead>
<tr>
<th>DNA polymerase</th>
<th>α</th>
<th>β</th>
<th>γ</th>
<th>δ</th>
<th>ε</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zidovudine</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>[21,23]</td>
</tr>
<tr>
<td>Lamivudine</td>
<td>−</td>
<td>+</td>
<td>+/−</td>
<td>−</td>
<td>−</td>
<td>[26,103]</td>
</tr>
<tr>
<td>Stavudine</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>[27]</td>
</tr>
<tr>
<td>Zalcitabine</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>[23,24]</td>
</tr>
<tr>
<td>Didanosine</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>[104]</td>
</tr>
<tr>
<td>Abacavir</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>[7]</td>
</tr>
<tr>
<td>Nevirapine</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>[31]</td>
</tr>
<tr>
<td>Adefovir</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>[34,105]</td>
</tr>
</tbody>
</table>

Note: the individual Kᵢ or Kᵢₒ data were not comparable between the different assay systems used and therefore not included. +, Inhibitory interaction demonstrated; +/−, interaction demonstrated, but not inhibitory; −, no interaction demonstrated.
Adverse mitochondrial effects of RTI

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With the exception of complex II, which is encoded entirely by nuclear DNA (nDNA), the other respiratory chain complexes I, II and IV and complex V are encoded both by nDNA and extrachromosomal mtDNA (Fig. 2). mtDNA consists of a double-stranded circular DNA molecule composed of 16569 base pairs, coding for 22 transfer RNA, two ribosomal RNA, and 13 subunits of the oxidative phosphorylation system. mtDNA can be replicated, transcribed and translated independently of nDNA metabolism. However, cell function and mitochondrial function are interdependent [36,37]: for replication of mtDNA the nuclear-encoded DNA polymerase \( \gamma \) is needed (see above). nDNA controls the synthesis of 90–95% of all mitochondrial proteins [38,39]; mtDNA is therefore semiautonomous.

There are several differences in structure and function between nDNA and mtDNA. At first, mtDNA is predominantly maternally inherited. The DNA of mitochondria is directly inherited from the cytoplasm of mainly the oocyte; less than 0.1% of the mtDNA is contributed by the sperm [36,38,40]. Second, mtDNA does not recombine and undergoes replicative segregation during both mitosis and meiosis. Each human cell contains hundreds of mitochondria and each mitochondrion contains two to 10 mtDNA molecules. When a cell divides, both mutated and non-mutated forms of mtDNA are randomly segregated into the daughter cells, resulting in mixtures of mutant and wild-type mtDNA in cells and human lineages [37–39,41]. Due to this coexistence of mutant and wild-type mtDNA, called heteroplasmy, otherwise lethal mutations can persist [36]. The severity of a defect due to mtDNA mutation depends on the nature of the mtDNA mutation and on the proportion of mutant mtDNA within the cell; mtDNA mutations will result in cellular malfunction when a certain threshold is reached, a phenomenon called threshold expression [37–39]. This expression depends on the severity of the oxidative phosphorylation defect and the relative reliance of each organ system on mitochondrial energy production. Mitochondria replicate more often than nuclei, and therefore the relative proportion of mutant and wild-type mtDNA may change within a cell cycle [38,39]. More replications indicate a larger chance to develop replication abnormalities. Because mtDNA has no introns, a random mutation will usually strike a coding DNA sequence. Furthermore, mutations and defects can easily occur because mtDNA has neither an effective repair mechanism nor protective histones, and it is exposed to oxygen radicals generated by the respiratory chain [16,36,38,40,42].

Altogether, mtDNA appears to be extremely vulnerable to genetically and exogenously acquired mutations. Since DNA polymerase \( \gamma \) appears to be the only regulating enzyme of mtDNA replication, inhibition of this enzyme with RTI might easily downregulate this replication resulting in decreased mitochondrial energy generation.

**Oxidative phosphorylation disorders**

Genetically inherited defects in mtDNA or nuclear genes encoding the oxidative phosphorylation system, leading to an impaired oxidative phosphorylation, give rise to a variety of clinical diseases due to failure in ATP synthesis [36,37,39,41,43–45]. This failure can affect virtually all organ systems, but tissues with the highest energy demand are most susceptible [38,46]. Disease symptoms will appear when the mitochondrial energy-generating capacity will fall below the energetic threshold of an organ [36,37,41,44]. Many organ systems have been described to be possibly affected (Table 2): liver, pancreas, heart, skeletal muscle, nervous system, haematopoietic system, inner ear, kidney.

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Manifestations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neurological</td>
<td>Peripheral neuropathy, encephalopathy, dementia, seizures, stroke</td>
</tr>
<tr>
<td>Myopathy</td>
<td>Hypotonia, muscle weakness, exercise intolerance</td>
</tr>
<tr>
<td>Cardiac</td>
<td>Cardiomyopathy, conduction disorders</td>
</tr>
<tr>
<td>Endocrine</td>
<td>Diabetes mellitus</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>Colonic pseudo-obstruction, exocrine pancreas dysfunction, pancreatitis, hepatomegaly, steatosis, liver failure, lactic acidosis</td>
</tr>
<tr>
<td>Nephrological</td>
<td>Non-selective proximal tubular dysfunction with acidemia, phosphaturia and glucosuria, glomerulopathy</td>
</tr>
<tr>
<td>Haematological</td>
<td>Anaemia, thrombocytopenia, pancytopenia</td>
</tr>
<tr>
<td>Psychiatric</td>
<td>Depression</td>
</tr>
<tr>
<td>General</td>
<td>Multiple systemic lipomas, fatigue</td>
</tr>
</tbody>
</table>
renal failure is rare), and eye. Liver cells and pancreatic \( \beta \) cells are highly dependent on oxidative metabolism and are therefore easily vulnerable to energy depletion, leading to liver disease and diabetes. Other (genetically inherited) clinical manifestations encountered in mitochondrial cytopathies are blindness, deafness, dementia, movement disorders, weakness, cardiac failure, and renal dysfunction [36,43,44,47].

The amount of mtDNA defects is one of the principal factors that determines whether a defect is expressed clinically. Usually the highest levels of mtDNA defects are in post-mitotic tissues such as skeletal muscle. Lower levels are seen in rapidly dividing tissues such as blood. Tissues with a slow turnover of mtDNA accumulate the largest number of mtDNA defects [36,44]. When a certain threshold is reached after accumulation of mtDNA defects, a deficient production of ATP with its consequences for a specific tissue will emerge. As deficient oxidative phosphorylation increases with mitochondrial damage, mitochondrial ATP production declines until it falls below the minimum energy levels (threshold expression) necessary for oxidative tissues and organs to function [16,37].

A disturbed function of the oxidative phosphorylation system will give rise to an altered oxidoreduction status (Fig. 3): a disturbed redox state (increased NADH/NAD\(^+\) ratio) shifts the pyruvate/lactate equilibrium in the direction of lactate and leads to a functional impairment of the Krebs cycle. 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\(^+\) is required for the adequate metabolism of glycolytic substrates [45]. Similarly, a postprandial increase of ketone bodies synthesis can be observed, related to the channelling of acetyl-coenzyme A towards ketogenesis [48]. Fat (triglycerides and free fatty acids) will accumulate intracellularly, which can be demonstrated histologically (macrovesicular hepatic steatosis).

In electron microscopy, histological damage can be demonstrated as swollen enlarged mitochondria, with or without loss of cristae, matrix dissolution, lipid droplets, paracrystalline and scattered vesicular inclusions [16,46,47,49,50].

**Nucleoside analogues and mitochondrial toxicity**

Apart from the inheritable route, mtDNA defects can also be acquired exogenously by toxic agents such as alcohol, tobacco and drugs [16,36,47]. In the latter group, drugs that have been shown to induce mitochondrial toxicity are nucleoside analogues used in chemotherapy and antiretroviral therapy, such as HIV RTI (as discussed in this review), but also cytarabine, vidarabine, aciclovir, and ribavirin. Since these nucleoside analogues elicit complete mtDNA replication deficits, clinical features can be regarded as a compilation of those seen in the genetic mitochondrial cytopathies. These features include myopathy, cardiomyopathy, neuropathy, lactic acidosis, exocrine pancreas failure, liver failure and bone-marrow failure [16,44,46,51–54].

A classical example of a drug with this mitochondrial toxicity is fialuridine. A trial with this nucleoside analogue in patients with chronic hepatitis B infection ended after 13 weeks due to severe adverse events. Hepatic failure, lactic acidosis, pancreatitis, neuropathy and myopathy due to mitochondrial toxicity were found and even persisted after discontinuation of the drug. Five of the 15 patients died due to one or more of these serious adverse events [42,47,55]. Fialuridine seriously decreased the abundance of mtDNA in cultured hepatoblasts [56], which was also seen in fialuridine-treated woodchucks, although the degree varied in the different tissues. The decline in mtDNA was 55% in heart, 65% in kidney, 74% in liver and 87% in muscle tissue [46]. Fialuridine is not a dideoxynucleoside analogue (unlike RTI) and has a different mode of action from RTI [57]. Although it is beyond the scope of this review, this drug clearly shows the consequences of inducing defective mtDNA replication by

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**Fig. 3.** Schematic presentation of pyruvate oxidation pathway leading to ATP production. When oxidative phosphorylation function is interrupted, ATP production will decline and the NADH/NAD\(^+\) ratio will rise, followed by (i) impairment of the flux through the Krebs cycle, (ii) channelling of acetyl-coenzyme A (CoA) towards ketogenesis, (iii) lactic acidemia, and (iv) an increased lactate/pyruvate ratio. OMM, Outer mitochondrial membrane; IMM, inner mitochondrial membrane; LDH, lactate dehydrogenase; PDHc, pyruvate dehydrogenase complex; FADH\(_2\), reduced form of flavin adenine dinucleotide; ANT, adenine nucleotide translocator.
nucleoside analogues [58]. In this light, it is not surprising that RTI have also been demonstrated both \textit{in vitro} and \textit{in vivo} to induce mitochondrial toxicity.

\textbf{In vitro}

Mitochondrial toxicity of antiretroviral nucleoside analogues was initially confirmed \textit{in vitro} studies. Studies with nucleoside analogues in human T-lymphoblastoid cell lines (CEM and MOLT-4F) [29,59,60] demonstrated that within a few days the antiretroviral drugs decreased the mtDNA content of the cells (Fig. 4a). There was a significant difference compared with control cells. Furthermore, morphological changes in mitochondria were found, such as enlargement of the mitochondria and fragmentation of the cristae. Increased lactic acid production was observed as a result of this damage (Fig. 4b) [29,46]. Although mitochondrial damage will result in an increase of alternative energy production (glycolysis), the capacity of the individual drugs to enhance lactic acid production was not directly correlated with their inhibition of mitochondrial synthesis [29,36]. The potency to inhibit the mtDNA content varied between the several agents: the concentration required to reduce the mtDNA content of CEM cells by 50\% after a 4-day drug treatment was 0.022 µmol/l for ddC, 3 µmol/l for D4T, 19 µmol/l for ZDV and 290 µmol/l for ddl [29]. (In comparison, the therapeutic concentration of ZDV, ddl and D4T is ± 4 µmol/l, and of ddC ± 0.04 µmol/l.) Long-term exposure of a pancreatic cell line (BxPC-3) to 10 µmol/l ddl for 18 days both impaired cell growth with lactic acid production and increased numbers and size of cytoplasmic lipid droplets with abnormal mitochondria [61]. In a nerve growth factor-primed cell line (PC-12) both ddC, D4T and ddl inhibited neurite regeneration in a dose-dependent fashion, whereas ZDV and 3TC had no influence, although this could not be attributed to a decrease of mtDNA for all drugs [62]. Finally, ZDV, ddl and ddC induced mitochondrial toxicity in cultured human muscle cells, with decreased cell proliferation and differentiation, increased lactic acid production, lipid droplet accumulation and impaired activity of respiratory chain enzymes [63]. No data of \textit{in vitro} mitochondrial toxicity have been reported to date for abacavir or adefovir.

The diversity in the severity of toxicity in the different tissues could be related to the variation in cell division and (mt)DNA production. Chen \textit{et al.} [29] studied the effect of cell differentiation and sensibility to drug-induced mitochondrial toxicity. They found an increase in mtDNA content after differentiation of proliferating cells into non-proliferating neuron-like cells (PC12 rat cells) [29]. A preferential effect by antiretroviral nucleoside analogues on mtDNA in quiescent cells is based on the higher turnover rate of mtDNA compared with nDNA [29].

These studies demonstrated that there is a difference in the drug concentration necessary for cell growth inhibition and inhibition of mtDNA synthesis. Higher dosages are required to inhibit mtDNA synthesis [29]. Furthermore, it could be demonstrated that mtDNA content is a significantly more sensitive measure of antiretroviral nucleoside analogue toxicity than cell viability and mitochondrial morphology [60].

\textbf{In vivo}

Many adverse effects of RTI have been recognized (Table 3) and some of these include events caused by mitochondrial dysfunction.

Myopathy in long-term therapy with ZDV due to mitochondrial damage has been described by several investigators [64–68]. Histological features of ragged-red fibres (subsacrolemmal proliferation of abnormal mitochondria) have been demonstrated [66]. This...
Destructive mitochondrial myopathy appears to be reversible since some patients have shown a substantial reduction in ragged-red fibres and a concomitant pronounced increase in muscle mtDNA after discontinuation of ZDV [64,65]. To distinguish HIV-related myopathy from ZDV-induced myopathy, histochemical, immunocytochemical and electron microscopic features of muscle biopsies were obtained [67]. The typical ragged-red fibres were found in the biopsy specimens of the patients treated with ZDV and not in the untreated HIV-infected patients. Furthermore, remarkable histological improvement was seen in the biopsy specimens of patients in whom myopathy responded to discontinuation of ZDV [68].

A 5-year follow-up study with ddI among 72 patients exposed pancreatitis and peripheral neuropathy as the most important clinical toxicities of this agent [69]. The onset of these symptoms occurred after several weeks of treatment. Pancreatitis was observed after 10–18 weeks of therapy and peripheral neuropathy was observed after 18 and 30 weeks. No haematopoietic toxicity with ddI was found and pre-existing thrombocytopenia even improved after ddI was started. Development of peripheral neuropathy or pancreatitis has also been seen in ddC and D4T therapy [3,4,6,70,71]. Rabbits treated with ddC developed dose-limiting neurotoxicity. Mitochondrial alterations were demonstrated in Schwann cells of striatic and tibial nerves and dorsal root ganglia and the complex aggregation of mitochondria observed was thought to be an adaptive response to the ddC-induced mitochondrial dysfunction [72]. Many clinical studies have confirmed the occurrence of peripheral neuropathy during NRTI treatment [73–76], and in one report the ddC-induced neuropathy worsened when treatment was switched to ddI, suggesting a synergistic interaction of the two agents [77].

Hepatic steatosis and fulminant hepatitis are signs of severe toxicity in long-term antiretroviral therapy. Fatal outcome due to this hepatic steatosis with severe lactic acidosis has been reported for several NRTI (ZDV, ddI and D4T) after several months of treatment. Histological examination displayed macrovesicular steatosis (Fig. 5) and marked periportal intrahepatic cholestasis without features of malignancies or infectious agents [25,49,51,53,54,78–81]. This clinical ‘syndrome’ often starts with complaints of nausea, vomiting, weakness, abdominal pain and diarrhea. Later on, malaise, anorexia and dyspnoea may occur, and rapid progression to fatal acidosis may follow [82]. In one retrospective study, the incidence of this syndrome in a cohort of antiretroviral users was calculated to be 1.3 per 1000 person-years of follow-up [52].

Bone-marrow toxicity has been demonstrated for ZDV, but ddC, ddI and D4T also show in vitro toxicity to haematopoietic progenitor cells [83–87]. The mechanism of this toxicity appears to be multifactorial, in which both ddNTP and their metabolites seem to be involved [88,89]. ZDV seems to inhibit the mitochondrial haem synthesis, most likely through the inhibition of DNA polymerase γ [90], and it has been suggested

### Table 3. Adverse events of reverse transcriptase inhibitors.

<table>
<thead>
<tr>
<th>Type</th>
<th>Zidovudine</th>
<th>Lamivudine</th>
<th>Stavudine</th>
<th>Zalcitabine</th>
<th>Didanosine</th>
<th>Abacavir</th>
<th>Nevirapine†</th>
<th>Adefovir</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neuropathy</td>
<td>–</td>
<td>–</td>
<td>++</td>
<td>++</td>
<td>ID</td>
<td>–</td>
<td>ID</td>
<td></td>
</tr>
<tr>
<td>Myopathy</td>
<td>+/–</td>
<td>+/–</td>
<td>–</td>
<td>–</td>
<td>ID</td>
<td>–</td>
<td>ID</td>
<td></td>
</tr>
<tr>
<td>Cardiomyopathy</td>
<td>+/–</td>
<td>–/–</td>
<td>–</td>
<td>–</td>
<td>ID</td>
<td>–</td>
<td>ID</td>
<td></td>
</tr>
<tr>
<td>Pancreatitis</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>ID</td>
<td>–</td>
<td>ID</td>
<td>+</td>
</tr>
<tr>
<td>Hepatic steatosis/hepatitis</td>
<td>+/–</td>
<td>+/–</td>
<td>++</td>
<td>–</td>
<td>ID</td>
<td>–</td>
<td>ID</td>
<td></td>
</tr>
<tr>
<td>Lactic acidosis</td>
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<td>+/–</td>
<td>–</td>
<td>+</td>
<td>ID</td>
<td>–</td>
<td>ID</td>
<td>+/–</td>
</tr>
<tr>
<td>Nephrologic toxicity</td>
<td>+/–</td>
<td>+/–</td>
<td>–</td>
<td>+</td>
<td>ID</td>
<td>–</td>
<td>ID</td>
<td></td>
</tr>
<tr>
<td>Bone-marrow toxicity</td>
<td>+/–</td>
<td>+/–</td>
<td>–</td>
<td>+</td>
<td>ID</td>
<td>–</td>
<td>ID</td>
<td>+/–</td>
</tr>
<tr>
<td>Skin toxicity</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>ID</td>
<td>–</td>
<td>ID</td>
<td>+/–</td>
</tr>
</tbody>
</table>

References: [2,49,51,52,64,97,99], [5,102,106], [6,29,80,81], [4,59,97,107], [3,25,52,78,92,93,97,99], [108].

*Written information from the manufacturer, Glaxo-Wellcome plc, UK. †Written information from the manufacturer, Gilead-Sciences Inc., USA. ‡Insufficient data exist about the toxicity of other non-nucleoside analogues as delavirdine or efavirenz. +++, Most prominently observed toxicity; +, observed toxicity; +/-, observed possible toxicity; –, toxicity not observed; ID, insufficient data (most often from ongoing Phase II/III trials).

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Fig. 5. Macrovesicular hepatic steatosis in a patient treated with zidovudine and lamivudine [102].
that the supplementation of hemin might overcome this toxicity [86,90]. ZDV causes macrocytosis in almost every patient [91] and can induce anaemia and neutropenia in up to 50% of treated patients, depending on the daily dosage given [91]. Furthermore, it can produce a loss of haematopoetic precursors in peripheral blood in a time- and dose-dependent fashion [16]. Thrombocytopenia has been observed in patients using dDC [59] and ddI [92,93].

Although cardiomyopathy is a recognized feature in HIV-infected individuals [94,95], an association with the use of RTI has not been extensively investigated. Apart from ZDV-induced mitochondrial toxicity in rat hearts [96], Herskowitz et al. [97] reported six patients with cardiomyopathy out of 13 who received RTI therapy with ZDV, dDC or ddI [97]. Improvement occurred in four patients after discontinuation of therapy, but further depression of cardiac function was seen after ZDV was switched to either ddI or ddC. Domanski et al. [98] found a similar causal relationship in ZDV-treated children, but were unable to demonstrate this for ddI.

Nephrological toxicity has not been reported for any NRTI or non-NRTI, but during the 12th World AIDS conference in Geneva (June 1998) Jim Rooney (from Gilaed-Sciences Inc., USA) presented the most recent safety profile of adefovir: after 48 weeks of treatment a substantial percentage of patients developed some degree of renal tubular acidosis, in many cases accompanied by hypophosphatemia [108]. Although the etiology of this toxicity has to be worked out in further detail, this observation strongly resembles the proximal tubular dysfunction seen in oxidative phosphorylation disorders (Table 2).

Discussion

As shown above, there is substantial evidence that mitochondrial toxicity is the culprit in the adverse effects of NRTI therapy. A major problem with this toxicity is its time dependency and therefore delayed onset. Multi-organ side-effects are seen in long-term therapy (several months) with nucleoside analogues. In some cases, reversal of symptoms was obtained after cessation of the drugs, and in others, toxicity persisted despite drug discontinuation, occasionally with a fatal outcome [16,29,36,49,52–54,99].

Since NRTI are likely to remain the cornerstone of combination antiretroviral therapy, this toxicity might seriously hamper the success of this treatment. Early recognition to prevent irreversible damage seems warranted, but to date possible risk factors that contribute to the development of these side-effects have been poorly defined.

In one report it was suggested that female gender, obesity and HIV status (absence of AIDS-defining illness at moment of toxicity) are possible risk factors [49], but another report could not confirm this observation and showed that both men and women, and patients in early and late stages of HIV infection appeared to be susceptible to adverse events [52]. However, it remains intriguing that only some individuals appear to be susceptible to the development of toxicity, even after a short period of time, which is probably explained by a certain genetic susceptibility. Interindividual polymorphism of DNA polymerase γ with different affinities for NRTI might play a role, but this also applies for the numerous polymorphisms of mtDNA itself. Although a patient’s tissue metabolism might appear to function quite normally, the threshold expression of energy deficit might be much lower due to a genotypically different oxidative phosphorylation system. These persons will be more susceptible to the downregulation of mtDNA by NRTI. This mechanism was demonstrated in vivo in fibroblasts derived from patients with Kearns–Sayre syndrome, a neuromuscular disorder caused by a 4977 base-pair deletion in mtDNA. In a mixed population of cells, nucleoside analogues (ZDV, dDC) increased the mean levels of mutated mtDNA while decreasing the levels of wild-type mtDNA, presumably by preferentially inhibiting the proliferation of cells with little or no mtDNA mutations [100]. Since mtDNA is highly polymorphic, with a strong dissociation between genotype and phenotype [47], mtDNA polymorphism may play a central role in the observed interindividual susceptibility for NRTI toxicity. As with the increasing expression of inherited mtDNA abnormalities during ageing [37], susceptibility for NRTI toxicity might furthermore increase in older patients. To date, no data are available on these issues.

Apart from these epidemiological mysteries, another unsolved problem is the fact that some patients develop certain adverse events, whereas other patients using the same drug develop quite different adverse events. Furthermore, why do patients not always develop mild adverse events first, followed by more severe events? Not all nucleoside analogues cause the same side-effects (Table 3) and there appears to be a tissue selectivity in nucleoside analogue toxicity [16]. This tissue selectivity may be related to differential phosphorylation of antiretroviral nucleoside analogues or specificity of cellular kinases for phosphorylation of nucleoside analogues in different tissues [16]. In addition, most tissues have cell cycle-specific enzyme activities. This difference in metabolism may play a role in the differences in which the nucleoside analogues are transformed to their active metabolites. Moreover, there may be a distinction in
mitochondrial growth between proliferating and non-proliferating cells. Both issues can contribute to the tissue specificity (and also delayed onset) of adverse events. All these factors have been condensed in the so-called ‘polymerase γ hypothesis’ [16,59], so named because of the pivotal role of DNA polymerase γ in this process. It states that the toxicity of antiretroviral nucleoside analogues depends on (i) the subcellular availability and abundance of the antiretroviral nucleoside analogues in the target tissue; (ii) the ability of cellular nucleoside kinase to use the antiretroviral nucleoside analogue as a competitive alternative substrate resulting in monophosphorylation and later triphosphorylation of the antiretroviral nucleoside analogues; (iii) the ability of the triphosphate of the antiretroviral nucleoside analogue to inhibit DNA polymerase γ either by serving as a competitive (ineffective) alternative substrate or by chain termination of the nascent mtDNA strand (non-competitive); and (iv) the metabolic reliance on oxidative phosphorylation in the target tissues [15,16,38,59,101].

Conclusion

Mitochondrial toxicity is a clearly recognized adverse effect of NRTI. The clinical features of this toxicity, which can be both reversible and irreversible, vary inter-individually and between several tissues. In some cases fatal outcome can occur. Additional studies should be performed to determine which factors play a role in the predisposition to develop this toxicity. Insight in the seriousness of this problem is lacking and diagnostic tests that can determine the development of this toxicity at an early phase are not yet available. Clinicians should be aware of this toxicity in the management of long-term treatment with NRTI since the toxicity hazards of these drugs might easily outgrow the success of antiretroviral therapy.

Acknowledgements

The critical reading of the manuscript by Prof. J. Veerkamp, E. Mariman and R. Wevers was highly appreciated.

References


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