

Nucleoside analogues and HIV: the combined cost to mitochondria

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Keywords: nucleoside analogue, mitochondria, HIV

Highly active antiretroviral therapy (HAART) has revolutionized human immunodeficiency virus (HIV) care in the developed world. Since the introduction of HAART the morbidity and mortality associated with HIV infection have decreased dramatically,¹ but this benefit has been associated with the emergence of a diverse range of antiretroviral toxicities.

Nucleoside reverse transcriptase inhibitors (NRTIs) are central to effective HAART, with current antiretroviral guidelines recommending the inclusion of at least two of these agents in each regimen. However, NRTIs also contribute significantly to the toxicities of HAART. Here we review the known mechanism of action of NRTIs, the proposed mechanism by which they may cause toxicities through mitochondrial damage, the evidence that HIV infection may predispose individuals to overt mitochondrial dysfunction, and potential future methods of monitoring and preventing these problems.

Nucleoside analogues act as alternative substrates for DNA polymerases. Because NRTIs lack a hydroxyl group in the 3'-position (required for the addition of the next nucleotide onto the primer) their incorporation causes termination of the growing DNA strand. Their intended therapeutic action is to prevent the formation of HIV DNA by inhibiting HIV reverse transcriptase. However, NRTIs have the potential to cause serious cellular toxicities by interacting in a similar manner with human DNA polymerases.²

The enzymes responsible for the replication of human nuclear DNA are polymerase α and δ . Polymerase β and ϵ play roles in DNA repair, and polymerase γ is responsible for replication of mitochondrial DNA (mtDNA). The NRTIs currently used in the treatment of HIV infection (zidovudine, stavudine, lamivudine, didanosine, zalcitabine and abacavir) all inhibit DNA polymerases β and γ . The inhibition of polymerase β has not been shown to be of clinical significance, but

it is widely believed that many side effects of NRTIs stem from their effect on polymerase γ and the likely resulting mitochondrial dysfunction.²⁻⁵

Inherited abnormalities of mitochondria are associated with a diverse range of clinical syndromes. Features common to many such disorders include prominent involvement of the central and peripheral nervous systems, myopathies (including cardiomyopathies), bone marrow disorders (particularly anaemia) and metabolic derangements including pancreatic dysfunction.⁶ Similarly nucleoside analogues have been associated with the development of metabolic abnormalities, including asymptomatic hyperlactataemia, lactic acidosis and hepatic steatosis. Mitochondrial myopathies, including cardiomyopathy, are described with zidovudine exposure. Other recognized complications of NRTI therapy include macrocytosis (zidovudine and stavudine), peripheral lipotrophy (particularly with stavudine), sensory neuropathy (zalcitabine, stavudine and didanosine) and pancreatitis (didanosine and stavudine).

In vitro studies have demonstrated a hierarchy of NRTIs in terms of their ability to inhibit polymerase γ . Zalcitabine is the most potent inhibitor of this enzyme, followed by didanosine and stavudine, with zidovudine, lamivudine and abacavir all being relatively weak inhibitors of mtDNA synthesis.⁷ This is independent of their potency of inhibition of HIV reverse transcriptase, with zidovudine and stavudine being approximately equivalent in this regard.⁵

Some side effects attributed to mitochondrial dysfunction occur predominantly with exposure to those NRTIs that inhibit polymerase γ most strongly—for example, lactic acidosis, pancreatitis, lipotrophy and sensory neuropathy. However, other toxicities, including zidovudine-associated myopathy, are associated primarily with drugs that are relatively inefficient inhibitors of mtDNA synthesis. This apparent dilemma is most likely explained by the complex

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Leading article

pharmacology of NRTIs. Nucleoside analogues are prodrugs that can only act as substrates for DNA polymerases following stepwise intracellular metabolism to their active, triphosphorylated form.⁸ Hence the efficacy and potential toxicities of NRTIs are dependent on their metabolism within tissues rather than just the distribution of the parent drug.

Analogues of the various nucleosides are metabolized by different cellular kinases. Furthermore, any of the phosphorylation steps required to form the active drug may be rate limiting, and the affinity of enzymes for each agent may vary. Complex positive and negative feedback loops, not all of which have been characterized, regulate these phosphorylation pathways.⁸ In addition, NRTI metabolism is influenced by the cell type and whether the cell is activated or resting. For example, cytosolic thymidine kinase-1, an enzyme with high affinity for both zidovudine and stavudine, is expressed only during the S-phase of the cell cycle and is intrinsically absent from some cell types, including peripheral blood mononuclear cells (PBMCs).^{7,8} It is therefore expected that the levels of active metabolites of a given NRTI will vary in different tissues, and that within any given tissue the rates of metabolism of each compound will also vary.

Several groups are investigating the utility of quantifying cellular mtDNA in tissue from patients exposed to NRTIs as a possible tool for monitoring or diagnosing NRTI toxicities. An international quality assurance programme has confirmed that these assays give consistent and concordant results when utilized on the same sample.⁹ However, studies on tissue from clinical cohorts have yielded varying results. Early studies involving small numbers of patients demonstrated reductions in mtDNA in fat¹⁰ and buffy coat cells¹¹ from individuals with clinically significant NRTI toxicities. Several analyses of PBMCs have failed to show an association between mtDNA levels and current treatments or toxicities. Our own results suggest that mtDNA depletion may be a function of current NRTI exposure, and not obviously related to the presence or absence of side effects.¹² Notably, we have shown in >160 paired tissue samples from 62 subjects that different NRTIs may influence mtDNA levels in different cell types, with stavudine having the greatest effect in subcutaneous fat, and didanosine in PBMCs.¹³ This is consistent with the tissue-specific metabolism of NRTIs resulting in differential effects of these drugs in various cell types.

The effect of any NRTI on a given tissue is clearly more complex than can be explained solely by the ability of that compound to inhibit polymerase γ . In addition to tissue-specific metabolism (and hence effects) of NRTIs, HIV infection may also affect various tissues so as to predispose individuals to NRTI-induced mitochondrial toxicity.

In inherited disorders of mitochondrial function, 'thresholds' exist such that severe symptoms occur only when a certain percentage of mitochondria are affected. The precise threshold required varies among tissues, being lower in

tissues with higher energy consumption, such as muscle and brain.⁶ HIV may cause mitochondrial dysfunction, either through mutations in, or depletion of, mtDNA. This could then render infected individuals at increased risk of reaching the 'threshold' for clinically relevant toxicities if exposed to further mitochondrial damage from NRTIs.

Both clinical and laboratory data point to the possibility of HIV-induced mitochondrial dysfunction. A series of seven cases of unexplained severe lactic acidosis in HIV-infected individuals was reported from San Francisco in the late 1980s, including three patients who were not taking antiretroviral agents when this syndrome developed.¹⁴ A mitochondrial myopathy has been reported in NRTI-naive patients with HIV infection.¹⁵ Increased astrocyte apoptosis (a process thought to be driven by mitochondrial dysfunction) has been associated with HIV dementia.¹⁶ Lymphocytes collected from individuals undergoing symptomatic HIV seroconversion have prominent mitochondrial alterations in addition to an increased tendency to undergo apoptosis compared with control cells.¹⁷ *In vitro* studies of lymphoblastoid and monocytoid cells acutely infected with various strains of HIV demonstrate prominent mitochondrial abnormalities.¹⁸

The effects of HIV infection on other tissues may also render the host intrinsically susceptible to NRTI toxicities. For example, HIV is known to cause peripheral nervous system damage, with ~30% of those with AIDS affected by sensory neuropathy in the pre-HAART era, and evidence of peripheral neuropathology seen at post-mortem in 100% of those dying with AIDS.¹⁹ Although the mechanism by which HIV causes peripheral nerve damage remains obscure, it is likely that the virus contributes to the very high rates of neuropathy observed in NRTI-exposed outpatients with HIV today.²⁰ Non-human primate data support this theory. No neuropathy was seen in healthy primates exposed to extraordinarily high doses of stavudine²¹ or zalcitabine,²² but three of six SIV-infected macaques developed obvious neuropathy within 2 weeks of exposure to lesser doses of zalcitabine.²²

Despite the fact that individuals with HIV infection may be particularly predisposed to their toxicities, NRTIs are currently an essential component of effective HIV therapy. The development of strategies for using NRTIs safely is therefore a priority. It is possible that mitochondrial toxicity might be treated or prevented through supplementation with micronutrients known to contribute to healthy mitochondrial function. Anecdotal reports of NRTI-induced neuropathy improving with L-acetyl carnitine administration,²³ and a case report of coenzyme Q₁₀ being used successfully to treat zidovudine-associated myopathy without zidovudine cessation (Franklin L. Rosenfeldt, Department of Cardiothoracic Surgery, Alfred Hospital, Melbourne, Victoria, Australia, personal communication), lend support to this possibility, but formal studies are lacking.

NRTIs are effective and relatively specific inhibitors of HIV replication. Together with other antiretroviral agents they have revolutionized HIV care in the developed world, saving countless lives both by improving the life expectancy of patients and through the prevention of mother-to-child transmission of virus. However, the use of NRTIs is confounded by a significant burden of toxicities. It is likely that the pathogenesis of many of these toxicities is drug-induced mitochondrial dysfunction, and infection with HIV may confer an increased susceptibility to this. Ongoing studies will clarify the utility of monitoring tissue mtDNA levels in individuals exposed to NRTIs. In particular, the predictive value of a fall in tissue mtDNA in terms of the development of clinical toxicities, and the ideal cell type in which to monitor this, require clarification. It remains a research priority to investigate formally possible strategies for the prevention and treatment of mitochondrial toxicity from these important drugs.

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