HIV and mitochondrial toxicity in children

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In the last 10 years, the enormous impact of combination antiretroviral (ARV) therapy on paediatric HIV-associated mortality and morbidity in well-resourced settings and its role in the prevention of mother-to-child transmission (MTCT) of HIV cannot be underestimated. However, it is thus inevitable that children with HIV-1 infection will be exposed to ARVs for an ever-increasing length of time throughout post-natal growth and development, and the cumulative toxicities are becoming progressively apparent. Evidence for nucleoside reverse transcriptase inhibitor (NRTI)-associated mitochondrial toxicity is seen in vitro, in animal models and in NRTI-exposed adults and children. Proposed mechanisms of NRTI mitochondrial toxicity include, among others, impairment of mitochondrial DNA (mtDNA) replication and acquisition of mtDNA point mutations. Alterations in the mtDNA synthesis potentially reduce the production of mtDNA-encoded respiratory chain subunits, resulting in impaired oxidative phosphorylation and mitochondrial dysfunction. NRTI-associated mitochondrial toxicity in children has varied presentations including lactic acidosis, pancreatitis, cardiomyopathy and neuropathy, which are comparable to NRTI-exposed adults and children with congenital mitochondrial disorders. In the prevention of MTCT, uninfected infants are exposed to an ever-widening range of ARVs, often from conception and throughout fetal life. Animal models demonstrate evidence of mitochondrial toxicity from perinatal NRTI exposure, but controversy continues as to the extent of mitochondrial effects in NRTI-exposed children. Paediatric studies assessing the impact of reduced exposure to NRTIs or the use of NRTIs with lower mitochondrial toxicity are urgently required. In an era of expanding treatment options, minimizing toxicities becomes an increasing possibility, indeed a necessity.

Keywords: nucleoside reverse transcriptase inhibitors, mitochondrial DNA, DNA polymerase γ, HIV, children

Introduction

The introduction of highly active antiretroviral therapy (HAART) in the management of paediatric HIV infection in 1997 has resulted in a substantial reduction in HIV-associated mortality and morbidity. However, many children with perinatally acquired HIV infection are exposed to antiretroviral (ARV) drugs throughout post-natal growth and development, and as in adult cohorts, the medium- and longer-term side effects are becoming increasingly apparent. Varied clinical presentations including lactic acidosis, neuropathy, cardiomyopathy, myopathy, lipoatrophy, pancreatitis and bone marrow suppression have been attributed to mitochondrial toxicity in children exposed to nucleoside reverse transcriptase inhibitors (NRTIs) and are comparable to those seen in children with congenital mitochondrial disease because of mitochondrial DNA (mtDNA) deletions and depletions. Evidence for mitochondrial toxicity as a proposed mechanism is reported from HIV infection itself, as well as from NRTI therapy, with the majority of clinical data from adult studies. The wide spectrum in clinical manifestations may reflect the wide variation in tissue distribution and heteroplasmy of mitochondria (the existence of genetic heterogeneity within populations of mitochondria). Tissues with a higher energy requirement (for instance, brain and muscle) are more likely to reach a critical threshold for symptomatic mitochondrial disease following mitochondrial toxicity. Within the NRTI class, individual drugs differ both in their ability to induce mitochondrial toxicity and in the tissue specificity of toxic effects. The possibility of mitochondrial dysfunction in uninfected infants perinatally exposed to ARVs for the prevention of mother-to-child transmission (MTCT) of HIV was first raised in 1999, following a report of encephalopathy and mitochondrial dysfunction in uninfected infants born to HIV-positive mothers. ARVs are extremely beneficial in helping to reduce rates of MTCT, and with 40 million people infected with HIV worldwide, women being affected disproportionately, an increasing number of infants will be perinatally exposed to ARVs in the coming years. Reducing the potential for mitochondrial toxicity, the long-term effects of which are uncertain, is of increasing importance.

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Pathogenesis

There is evidence for mitochondrial damage from HIV itself and following therapy with NRTIs, the mechanisms of which have recently been well described (see reviews\(^\text{5,6}\).) HIV infection causes an inflammatory milieu with raised levels of pro-inflammatory cytokines, such as tumour necrosis factor-\(\alpha\), that may inhibit mitochondrial function and promote cellular apoptosis.\(^\text{4}\) In vitro HIV virulence factors (HIV Tat and viral protein R) have been shown to cause mitochondrial damage and may induce cellular apoptosis via a mitochondrial pathway.\(^\text{4}\) Premature mitochondrial ageing is of particular concern for a paediatric population who currently have the prospect of a lifetime on HAART. Mitochondrial DNA (mtDNA) mutations accumulate over time and mtDNA repair mechanisms are more limited than nuclear DNA (nDNA) repair mechanisms.\(^\text{12}\) Depletion of the number of mtDNA copies per cell in peripheral blood mononuclear cells (PBMCs) is reported in treatment-naive adults with HIV, and skeletal myopathies, cardiomyopathies and lipoatrophy have been observed in such patients.\(^\text{13}\)

NRTIs inhibit human DNA polymerase \(\gamma\) impairing synthesis of mtDNA, resulting in mtDNA depletion observed \(\text{in vitro}\) in neurones, adipocytes, muscle and pancreatic cells. Depletion in mtDNA and increased mtDNA mutations may reduce synthesis of mtDNA-encoded protein subunits required for oxidative phosphorylation (OXPHOS). The resulting reduction in ATP production and increased reactive oxygen species have the potential for further mtDNA damage.\(^\text{14}\) NRTIs have to be imported into (and transported within) mitochondria and phosphorylated to active forms, competing with the natural nucleotide pool in mtDNA replication. Alterations in these processes may impact on mtDNA production.\(^\text{11}\) In a cardiac mouse model, overexpression of the mitochondrial transport protein deoxynucleotide carrier resulted in increased rates of mitochondrial toxicity following exposure to HAART.\(^\text{15}\)

The majority of children exposed to NRTIs do not exhibit clinical evidence of mitochondrial disease. Compensatory mechanisms that increase mtDNA and levels and transcription/post-transcription function have been demonstrated in adults treated with NRTIs and this is likely to be protective in patients on ARV.\(^\text{16}\) Why certain NRTI-exposed children develop symptomatic mitochondrial toxicity appears to be multifactorial and may include factors such as genetic predisposition and length and type of NRTI exposure.\(^\text{11,17}\) NRTIs vary in their capacity to inhibit DNA polymerase \(\gamma\) with stavudine and didanosine having greater effect than zidovudine, which in turn appears to be more potent than lamivudine, abacavir and tenofovir.\(^\text{18}\) Additionally, mitochondrial toxicity may be increased when, as is usual in HAART, two NRTIs are used in combination.\(^\text{19}\) Further understanding of the pathogenesis of mitochondrial toxicity in children may aid in the prevention of the long-term organ side effects of HAART.

Mitochondrial toxicity and in utero NRTI exposure

The significant benefits of ARVs in reducing MTCT of HIV-1 towards rates of \(<1\%\) are well established; however, concern over potential mitochondrial toxicity continues.\(^\text{20}\) Mice exposed \(\text{in utero}\) to zidovudine and lamivudine showed reduced numbers of cardiomyocyte mitochondria and abnormal echocardiography (ECHO) measurements at birth and persisting into adult life.\(^\text{21}\)

\(E\)rythrome\(\text{us pata}\)s monkeys exposed \(\text{in utero}\) to human equivalent doses of zidovudine and lamivudine euthanized at birth were demonstrated to have incorporated zidovudine into fetal mtDNA in myocytes, hepatocytes, renal and placental cells. They also had reduced mtDNA in cardiac, skeletal muscle and brain tissue with reduced OXPHOS activity in both cardiac and skeletal muscles.\(^\text{22}\) Monkeys euthanized at 1 year of age and similarly exposed to NRTIs in the third trimester of pregnancy and for 6 weeks after birth showed normal ECHO and OXPHOS activity, but significant mitochondrial morphological damage on electron microscopy (EM) with mitochondrial proliferation and increased levels of mtDNA when compared with healthy controls.\(^\text{23}\) The investigators in this study suggested that the compensatory proliferative response of abnormal mitochondria, although not causing any functional abnormality in early life, may do so later as ageing progresses, with depletion of normal mitochondria and expansion of damaged organelles. Reassuringly, structural and functional cardiac ECHO abnormalities have not been seen in human infants exposed perinatally to NRTIs; however, they were only monitored up to 14 months of age.\(^\text{24}\) Conversely, both human and monkey infants perinatally exposed to NRTIs do show similar abnormal mitochondrial structure (by EM) and mtDNA depletion in umbilical cord and cord blood with additional mtDNA depletion seen in placentas of women taking NRTIs during pregnancy.\(^\text{25,26}\) The long-term implications of such findings remain uncertain; however, in uninfected children exposed perinatally to NRTIs, mtDNA depletion in PBMCs has been shown to persist up to 2 years of age.\(^\text{27}\)

Although evidence from \(\text{in vitro}\) and animal studies is of concern, cohort studies of children perinatally exposed to ARVs offer conflicting pictures. In 1999, eight HIV-uninfected children from the French cohort exposed perinatally to zidovudine or zidovudine and lamivudine were reported to have mitochondrial dysfunction, two of whom died of encephalopathy.\(^\text{3}\) Further in-depth analyses of over 2600 NRTI-exposed prospectively followed children in this cohort found evidence of neurological symptoms and/or significant hyperlactataemia in 12 children with respiratory chain and/or histological mitochondrial abnormalities, resulting in an 18 month incidence of 0.26\% when compared with 0.01\% expected incidence in the general population for paediatric neuro/mitochondrial disease.\(^\text{29}\) Conversely, initial retrospective analyses of other large cohorts from the USA and Europe did not report an increase in deaths due to, or clinical manifestations of, mitochondrial disease associated with ARV exposure.\(^\text{29,30}\) A more recent retrospective analysis from the Paediatric AIDS Clinical Trials Group (PACTG) Study 219 suggested a possible association between first exposure to lamivudine or zidovudine/lamivudine in the third trimester and mitochondrial dysfunction.\(^\text{3}\) However, the retrospective nature of these studies limits the identification of NRTI-associated mitochondrial toxicity, the signs of which may be subtle and non-specific, as was illustrated in the prospective French study and further rigorous prospective studies are required.

Transient hyperlactataemia in ARV-exposed infants shortly after birth is well recognized with small studies reporting rates of abnormal lactates in up to 90\% of infants, but it is usually asymptomatic resolving within weeks, although there are very occasional reports of fulminant lactic acidosis and multi-organ failure.\(^\text{32,33}\) More persistent but of uncertain significance are the effects of perinatal exposure to ARVs on haematopoiesis. Follow-up of a European ARV-exposed but HIV-uninfected
leading article

Mitochondrial toxicity in HIV-infected children

The effects of NRTIs on mitochondrial structure and function in HIV-infected adults are well recognized; however, data are more limited in paediatric populations. In both groups, many studies have small numbers and are cross-sectional, with conflicting results that may in part be due to differing methodologies and to the tissue specificity of mitochondrial disease. Adults on NRTI-based HAART have been shown to have reduced mtDNA quantity and function in skeletal muscle with increased mtDNA deletions, although this did not correlate with reduced mtDNA in PBMCs. It is hoped that quantifying mtDNA as a ratio to nDNA in PBMCs will avoid invasive tissue biopsies, a particular problem in paediatric patients. However, both the tissue-specific nature of mtDNA depletion and the potential for platelet mtDNA contamination must be considered. Typically, quantification of mtDNA in PBMCs, such as by real-time PCR, is expressed as a ratio of mtDNA to nDNA; platelets having no nDNA should therefore be effectively removed from samples to achieve meaningful results. In symptomatic adults, NRTI-associated mtDNA depletion has been reported in both renal and liver biopsies; the patients with the lowest mtDNA quantification had elevated blood lactate levels. Additionally, adults with high serum lactates have been shown to have reduced PBMC mtDNA, associated with stavudine usage, and an increasing number of NRTIs, but reversible with NRTI cessation.

In response to NRTI-associated mitochondrial toxicity in adults, ‘NRTI-switch studies’ have been undertaken, and patients on HAART are switched from NRTIs with a greater likelihood of inducing mitochondrial toxicity, such as stavudine, to NRTIs considered to be less toxic, such as lamivudine. Sixteen patients on stavudine with lipatrophy switched to abacavir or zidovudine and subsequently showed improvements at 48 weeks in dual-wavelength X-ray absorptiometry scans, fat apoptosis, mtDNA (fat, skeletal muscle and PBMCs) and mitochondrial enzyme activity. In well-resourced settings, stavudine usage is avoided, where possible, because of high levels of associated toxicity; however, currently, it continues to be widely used in the first-line ARV therapy in resource-limited regions. Small non-comparative studies in adults of NRTI-sparing regimens, where NRTIs are avoided and HAART is based on the use of non-nucleoside reverse transcriptase inhibitors, protease inhibitors and rarely the fusion inhibitor enfuvirtide, suggest trends towards improved NRTI-associated toxicities, mtDNA quantification and enzyme function. Paediatric studies are extremely limited, but NRTI-sparing regimens may become increasingly feasible as new classes of ARVs become available. However, the time delay in the access of new ARVs to paediatric patients and the difficulties in dosing and formulating palatable suspensions mean that NRTIs are likely to remain the backbone of paediatric HAART for the foreseeable future. Treatment interruption studies in adults have been halted early due to an excess of clinical adverse events in patients off treatment. However, an earlier adult study showed an increase in the mtDNA in peripheral blood lymphocytes on cessation of HAART but only after treatment was interrupted for 6 months. The cost/benefit of HIV treatment interruption for children may be different from adults as they potentially face a lifetime of HAART with its cumulative toxicities, and the following satisfactory interim safety analysis, a pilot European paediatric, CD4-driven, treatment interruption trial (PENTA 11: www.pentatrials.org) continues. A European observational cross-sectional study of 477 children, median age nearly 10 years, with a median duration of ARV exposure of over 4.5 years, showed that a quarter had signs of peripheral lipoatrophy and/or central lipohypertrophy and a third had dyslipidaemia associated, as in adults, with female sex, duration of ARV therapy, and stavudine or protease inhibitor usage. However, children with or without lipodystrophy have shown comparable mitochondrial function and apoptosis in PBMCs to healthy controls, contrasting with reports of increased sensitivity to apoptosis in infected children from other groups, the latter suggesting a possible premature ageing of the immune system. Such conflicting data may be due in part to the difficulties in using PBMCs to assess mitochondrial toxicity and obtaining tissue biopsies from children. Prospective longitudinal studies are required in paediatric cohorts to further establish the impact of HAART and NRTIs, in particular, on mitochondrial function and allied toxicities.

Conclusion

The enormous benefit of HAART in the reduction of HIV-associated morbidity and mortality for children and in the prevention of MTCT cannot be underestimated. In countries where HAART has been widely available for a decade, attention has now turned to the management of the long-term toxicities associated with HIV and ARV therapy. Minimizing the accumulation of HAART-associated toxicities has become a priority, and strategies such as NRTI switch/sparing and treatment interruption studies require further consideration, particularly as new classes and drugs within existing classes of ARVs become available. For children potentially facing a lifetime on HAART, such strategies may have equal or even greater impact than in adult cohorts, and the continuation and further development of collaborative paediatric trials in this area are of great importance.

Transparency declarations

None to declare.
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