REVIEW ARTICLE

Drug Safety 2007; 30 (10): 845-859
0114-5916/07/0010-0845/$44.95/0

Mitochondrial Disorders among Infants Exposed to HIV and Antiretroviral Therapy

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Abstract

Although antiretroviral therapy (ART) is critical for preventing mother-to-child transmission of HIV, concern has been raised about the possibility that it may cause mitochondrial dysfunction in infants. There is adequate evidence for a mechanism by which exposure to nucleoside reverse transcriptase inhibitors (NRTIs) could lead to mitochondrial dysfunction; animal studies have shown evidence of mitochondrial dysfunction in the offspring of animals treated with NRTIs and mitochondrial disorders occur in adults treated with NRTIs. This systematic review synthesises the published research on mitochondrial dysfunction and disorders in infants exposed to HIV and antiretrovirals.

We found conflicting evidence regarding the possible association of *in utero* ART exposure with mortality and morbidity due to mitochondrial dysfunction. ART exposure *in utero* or postpartum was associated with persistent decreases in lymphocytes, neutrophils and platelets as well as an increased risk of transient lactic acidaemia, anaemia and mitochondrial DNA depletion, although these laboratory findings were generally not associated with clinical symptoms.

We conclude that large, prospective studies of HIV-exposed infants are needed to resolve the discrepant results regarding morbidity and mortality related to mitochondrial disorders, to ascertain the clinical significance of effects on laboratory values, to determine whether or not the incidence of mitochondrial disorders differs by regimen and to develop predictive models that might identify which infants are at the greatest risk. The challenges that remain to be addressed include the development of a sensitive but affordable screening algorithm in combination with specific diagnostic criteria; consistent collection of data on ART exposure and other risk factors, long-term follow-up of HIV-exposed but uninfected children and implementation in resource-limited settings.

1. Background

The outcome for children born to women infected with HIV changed dramatically with the introduction and widespread use in developed countries of antiretroviral therapy (ART) prophylaxis.[1] ART for the prevention of mother-to-child transmission is now being rolled out in resource-poor settings where 13–43 of 100 infants born to HIV-infected women would otherwise be vertically infected.[2] With ART prophylaxis, the majority of those children will remain uninfected.[3] There is no question of the benefit that this intervention provides in preventing a substantial proportion of vertical transmission of the virus. However, there are some unanswered questions regarding the effects of ART on a developing fetus. Concern was raised following a report of eight children exposed *in utero* to zidovudine alone (n = 4), zidovudine plus lamivudine (n = 2), or zidovudine, lamivudine and didanosine (n = 2), with clinical symptoms consistent with mitochondrial dysfunction after 4 months of age.[4] Mitochondrial dysfunction manifests itself in a wide variety of symptoms resulting from abnormal oxidative phosphorylation. After providing some background on mitochondrial dysfunction, we will present the results of a systematic review of the literature on the association between exposure to ART and mitochondrial disorders in HIV-exposed but uninfected children.

1.1 Effect of Nucleoside Reverse Transcriptase Inhibitors (NRTIs) on Mitochondrial DNA (mtDNA) Replication and Repair

Detailed reviews of the effects of nucleoside reverse transcriptase inhibitors (NRTIs) on mitochondrial DNA (mtDNA) have been published elsewhere,[5-7] but a few key points follow for the pur-

poses of providing context. Findings from *in vitro* studies indicate that NRTIs have a significant inhibitory effect on γ-polymerase and a modest but detectable interaction with β-polymerase. Inhibition of γ-polymerase, an enzyme required for mtDNA replication, results in decreased overall levels of mtDNA, potentially leading to mitochondrial dysfunction. As β-polymerase is responsible for repairing errors during DNA replication, inhibition of this enzyme could also lead to mtDNA mutations. Mitochondrial dysfunction may also originate from mutations in inherited mtDNA or nuclear DNA.

1.2 Background Rate of Mitochondrial Disorders in the General Paediatric Population

Due to the varied clinical presentation and difficulty obtaining a definitive diagnosis of mitochondrial disorders, few studies have been conducted that provide estimates of the incidence in the general paediatric population. Two studies from Scandinavia report similar estimates of the incidence of approximately 1 case in 10 000 in this population.[8,9] From the Northern Ostrobothnian region of Finland with approximately 150 000 children, 17 paediatric cases were identified over a period of 7 years with neurological symptoms associated with mitochondrial respiratory chain enzyme deficit.[9] In Gothenburg, Sweden, between 1984 and 1998, 32 children with neurological symptoms indicative of significant enzyme deficit were identified from a population of approximately 360 000 children aged <16 years.[8] Additionally, in British Columbia, Canada, approximately 3 children per 100 000 births have a respiratory chain-based, mitochondrial disease.[10] A nationwide prospective study conducted in Italy estimated the rate of primary lactic acidaemia in the paediatric population at 1 in 27 106.[11] Overall, these studies suggest that the prevalence of mitochondrial disorders ranges between 1 in 9000 and 1 in 33 000 in the general paediatric population.

1.3 Paediatric Diagnosis, Presentation and Prognosis of Constitutional Mitochondrial Disorders

Diagnosis of mitochondrial disorders constitutes a challenge to clinicians, especially because they can occur in children in whom clinical presentation and progression show enormous variation. Bernier et al.[12] modified the adult criteria for constitutional mitochondrial disorders proposed by Walker et al.[13] so that they would be appropriate for use in infants and children. The modified Walker criteria classify a patient as having a respiratory chain disorder if two of the major criteria (e.g. respiratory chain encephalomyopathy, >2% ragged red fibres in skeletal muscle, fibroblast ATP synthesis rates >3 standard deviations below mean) or one major plus two minor criteria (e.g. nuclear or mtDNA mutation of probable pathogenicity, at least one metabolic indicator of impaired respiratory chain function, fibroblast ATP synthesis rates >2–3 standard deviations below mean) are present.

Using the modified Walker criteria, Scaglia and colleagues[14] identified 113 children with definite mitochondrial disorders out of 400 evaluated. Of these, 13 (11.5%) cases were linked to specific inherited mtDNA mutations and 3 (2.7%) were linked to nuclear DNA mutations but the vast majority were considered sporadic. In total, 71% (n = 80) had a significant respiratory chain defect. The average age at presentation was 40 months. Although 40% (n = 45) of confirmed cases presented with cardiac disease based on Doppler echocardiography, 60% (n = 68) presented with predominantly noncardiac symptoms. Among those with a noncardiac phenotype, the most common symptoms were developmental delay (100%), ophthalmological symptoms (32%) and hearing loss (21%). Plasma lactic acid levels were elevated at least once in 60% of cases. The average age at death was 5 years 4 months. At 16 years of age, survival among those with cardiomyopathy was 18% compared with 95% among patients with noncardiac symptoms.

Table I. Clinical and laboratory features of nucleoside reverse transcriptase inhibitor-associated mitochondrial toxicity[21,28-31]

Tissue	Clinical manifestation(s)	Laboratory markers	Causative drug(s)
Nerve	Paraesthesia, distal pain, numbness, reduced reflexes, Schwann cell abnormalities, seizures, febrile seizures, peripheral neuropathy, acquired microcephaly, cranial nerves paresis, impaired cognitive development (for children aged >1y), cerebellar dysfunction and ataxia, motor disabilities, paraparesis, spasticity	None	d4T (ddC, ddI, 3TC to lesser extent)
Muscle	Proximal muscle weakness, fatigue, myalgia, wasting, ragged red fibres changes in muscular tone, hyper- and hypotonia, myopathy	↑Creatine kinase	ZDV
Liver	Anorexia, malaise, abdominal pain, nausea, vomiting, ascites, hepatomegaly, hepatic encephalopathy	Lactic acidosis: mild: 2–5 mmol/L, severe: >5 mmol/L ↑Liver enzymes ↑Anion gap ↓Bicarbonate	ZDV, d4T, ddI, ddC
Pancreas	Abdominal pain, vomiting, pancreatitis	↑Serum amylase	ddI mainly (3TC, ddC to lesser extent)
Heart	Dilated cardiomyopathy, accessory pathways, heart block	None	ZDV, ddC, ddI
Bone marrow and blood	Pancytopaenia	Anaemia	ZDV, ddI, IND
		Neutropenia	ZDV, RIT, DLV, NFV
		Thrombocytopaenia	ddI, IND, RIT, DLV, NFV, SQV
Kidney	Renal tubular acidosis	Hyperchloraemic metabolic acidosis with normal serum anion gap	ADV
Fat	Lipoatrophy	May be associated with lactic acidosis	d4T mainly, also others

3TC = lamivudine; **ADV** = adefovir; **d4T** = stavudine; **ddC** = zalcitabine; **ddI** = didanosine; **DLV** = delavirdine; **IND** = indinavir; **NFV** = nelfinavir; **RIT** = ritonavir; **SQV** = saquinavir; **ZDV** = zidovudine; ↑ indicates increased; ↓ indicates decreased.

1.4 Mitochondrial Effects of NRTI Use in HIV-Infected Adults and Children

HIV infection itself is associated with mtDNA reductions in peripheral blood mononuclear cells (PBMC) based on evidence from seroconverters before and after they became HIV-infected as well as comparisons of HIV-infected to HIV-uninfected individuals.[15,16] Initially, treatment of HIV-infected individuals with antiretrovirals may improve mtDNA levels in PBMCs by bringing viraemia under control,[17,18] but longer term use is associated with a variety of adverse events believed to result from mitochondrial damage (table I). These include lactic acidosis, peripheral neuropathy, myopathy, pancreatitis, hepatopathy (including hepatic steatosis) and lipoatrophy.[7,19-21] The incidence of symptomatic mitochondrial dysfunction has not been studied in large cohorts of HIV-infected children, but there have been reports of hepatic steatosis, lactic acidosis, myopathy and pancreatitis associated with antiretroviral treatment.[7,22,23] These toxicities are typically reversible upon discontinuation of the associated agent.[24-27]

1.5 Evidence of Mitochondrial Dysfunction in Non-Human Primates Exposed to NRTIs *in Utero*

Studies have been conducted in primates to quantify the effects of *in utero* exposure to NRTIs separately from the effects of exposure to HIV. Transplacental zidovudine exposure was found to have an effect on mitochondrial function of the offspring of *Erythrocebus patas* (common name: patas monkey).[32,33] In particular, alterations of oxidative phosphorylation complexes (i.e. significant decrease in the activity of complex I and significant increase in the activity of complexes II and IV) were found in mitochondrial extracts of brain, heart and

muscle.[32,33] Furthermore, mtDNA depletion occurred in a dose-dependent fashion in fetal cardiac and skeletal muscle,[32] whereas a slight dose-dependent degradation but not depletion was found in fetal brain mtDNA.[33] Morphological abnormalities, such as sarcomere disruption and/or swollen or small mitochondria, were found selectively in cardiac and skeletal myocytes from zidovudine-exposed patas monkeys,[32] whereas mitochondria morphological abnormalities were found in brain tissues.[33] Gerschenson and colleagues[34] have also reported on the effects of *in utero* exposure to human-equivalent doses of combination zidovudine and lamivudine in fetal patas monkeys. Zidovudine, but not lamivudine, was integrated into the DNA of multiple tissues (liver, kidney, skeletal muscle and placenta) of the three fetal monkeys exposed in this study. In heart and skeletal tissue, there were changes in mitochondrial morphology and significant depletion (≥50%) of mtDNA levels in exposed compared with unexposed animals. Too little mtDNA was present in heart and skeletal tissues to assess the extent of degradation. In brain tissue, mtDNA levels were depleted by ≥50% and degradation of mtDNA was observed in cerebellar but not cerebral tissues from exposed animals.

The mitochondrial effects of combination zidovudine and lamivudine appear to be more severe than those of zidovudine alone, although exposed animals reportedly functioned normally during the first year of life and echocardiograms did not reveal significant changes. The significance of these findings and their relevance to ART-exposed infants is not known.

1.6 Summary and Objective

There is evidence for a mechanism by which NRTI exposure could lead to mitochondrial dysfunction; animal studies provide evidence of mitochondrial toxicity in the offspring of animals treated with NRTIs, and mitochondrial disorders occur in adults treated with NRTIs. The purpose of this review is to summarise the published research on the association between ART exposure and mitochondrial disorders in HIV-exposed but uninfected infants and to provide a basis for further investigation.

2. Literature Search Methodology

We searched PubMed for English-language articles published between 1993 and 31 August 2005 using the keywords 'highly active antiretroviral therapy' or 'HAART' or 'antiretroviral therapy' in combination with the words 'pregnancy' or 'uninfected children'. The search term 'mitochondrial and HIV and children' was also used. Lastly, 'European Collaborative Study and HIV' and 'French Perinatal Cohort Study' were searched to capture all publications from these key research groups. Case reports, editorials, review articles, conference abstracts and studies in non-humans were specifically excluded.

Articles were reviewed for information regarding *in utero*, peripartum and postpartum exposure to antiretroviral drugs among uninfected infants born to HIV-infected women and the incidence of adverse events related to this exposure. Reference lists of papers included in the review were used to identify other articles for potential inclusion.

A total of 358 articles were identified from the combined searches. Of those, 339 did not provide details regarding the incidence of adverse events among HIV-uninfected infants exposed to ART. Nineteen articles are included in this review. The characteristics of these studies are presented in the supplementary material ['ArticlePlus'] available at http://drugsafety.adisonline.com.

3. Mortality Related to Mitochondrial Disorders

In the study that raised concern regarding possible mitochondrial effects of *in utero* ART exposure, Blanche et al.[4] reported two deaths, both among children exposed *in utero* to zidovudine plus lamivudine in France. Cohort studies of the relationship between ART exposure and mitochondrial disorders were initiated in response. Barret et al.[28] (French Perinatal Cohort Study) established that these two deaths arose from a cohort of 4426 HIV-exposed children, corresponding to a mitochondrial disorder-

related mortality rate of 4.5 cases in 10 000 children. Hankin and colleagues (European Collaborative Study, 2003)[35] reviewed 2414 uninfected children born to HIV-infected mothers of whom 42% (n = 1008) were exposed to ART. The authors did not find any evidence of mitochondrial disorders or an association between antiretroviral exposure and all-cause mortality.

The Perinatal Safety Review Working Group retrospectively reviewed the medical records of 223 HIV-exposed children who died before the age of 5 years from among 23 265 HIV-exposed infants in the US who were enrolled in one of five clinical trials or observational cohort studies.[36] The authors found no deaths that were likely to be due to mitochondrial disorders (one-sided, 97.5% CI 1.6/10 000). They reported three deaths among children with indeterminate HIV status where the differential diagnosis would have reasonably included mitochondrial disease (i.e. possibly due to mitochondrial dysfunction). Two were unexposed to ART. The third was not exposed after birth but lacked maternal medical records regarding possible *in utero* ART exposure. This child was born in 1991 and died at the age of 5 months from sepsis and pneumonia with comorbid hepatitis, cardiomyopathy and chronic respiratory illness. The overall mortality rate of 110 cases per 10 000 HIV-exposed children was consistent with the background rate in the US population (74 cases per 10 000 for White children and 172 cases per 10 000 for African American children in the paediatric population) given the over-representation of African American children in the combined cohort (55%). The authors also evaluated the possibility that ART exposure might lead to an increased risk of sudden infant death syndrome (SIDS) but found that the incidence of SIDS was consistent with the background rate in the US population.

4. Overall Morbidity Related to Mitochondrial Disorders

Drawing on the characteristics of children with constitutional mitochondrial disorders as well as the presentation of mitochondrial toxicity in NRTI-exposed adults, Barret et al.[28] (French Perinatal Cohort Study) conducted surveillance for probable cases of mitochondrial disorders based on the presence of unexplained neurological symptoms, significant abnormalities on MRI scan or hyperlactataemia among 4392 HIV-exposed but uninfected infants, 60% (2644) of whom were exposed to ART. This study reported an 18-month incidence of mitochondrial disorders for ART-exposed children of 0.26% (95% CI 0.10, 0.54). They found no cases of 'established' or 'possible' mitochondrial disorders among the remaining 40% (1748) of the cohort who were HIV-exposed but ART-unexposed children (21 of 2644 exposed vs 0 of 1748 unexposed; $p < 0.002$).

Bulterys et al.[37] (Perinatal AIDS Collaborative Transmission Study) examined the clinical, laboratory and hospitalisation records of 1954 HIV-negative children born to HIV-infected mothers for signs or symptoms that might suggest mitochondrial disorders. Thirty-three possible cases were further investigated by collection of standardised data from the paediatric clinical director at that site. Ultimately, one child was identified with symptoms consistent with mitochondrial disorders (epilepsy and developmental delay) and two children were identified with transient signs or symptoms for which mitochondrial disorder was considered possible but unlikely. None of the three children were exposed to antiretrovirals perinatally.

5. Cardiac Abnormality

Culnane et al.[38] (Pediatric AIDS Clinical Trials Group) observed similar rates of abnormal echocardiogram results in 186 children with and without prior ART exposure (16% in the zidovudine group vs 15% in the placebo group; $p = 0.84$). All of the children were asymptomatic. Lipschultz et al.[39] (Pediatric Pulmonary and Cardiovascular Complications of Vertically Transmitted HIV Infection Study Group) also reported no association between exposure to zidovudine and mean left ventricular fractional shortening at 10–14 months of age among 463 uninfected infants (39.0% in those exposed to zidovudine vs 38.1% among those never exposed to zidovudine; $p = 0.43$).

Bellón Cano and colleagues[40] reported six cases of cardiac defects among 126 neonates exposed to combination ART including persistence of ductus arteriosus, auricular septal defect, ventricular septal defect, pulmonary stenosis and aortic coarctation. Four were exposed to combinations without a protease inhibitor (PI) while two were exposed to a PI-based highly active antiretroviral therapy regimen. No details were given regarding first trimester exposure to antiretrovirals in these six cases, which also could have had teratogenic effects on the developing fetus. The authors report that 54 of 124 (44%) were exposed to heroin, cocaine or methadone during pregnancy but the specifics of exposure for these six infants are not provided. Unlike the reports by Culnane et al.[38] and Lipschultz et al.,[39] this study does not include an HIV-exposed but ART-unexposed comparison group making it difficult to determine whether this represents an elevated risk due to ART exposure.

6. Ophthalmological Abnormality

Culnane et al.[38] (Pediatric AIDS Clinical Trials Group) reported that there were three abnormal ophthalmological findings (two of astigmatism and one of ptosis) among 72 children exposed to zidovudine and one instance of epicanthal folds among 65 children exposed to placebo; however, the frequency of abnormal ophthalmological findings was not statistically different (Fisher's Exact test $p > 0.99$). In addition, there were two abnormal findings recorded on funduscopic examination among children in the zidovudine group: one instance of 'thinned vessels; discs looking slightly pale' and another of 'copper, beaten look' on the fundus.

7. Developmental Delay

Developmental delay is a non-specific symptom of mitochondrial deficits affecting the nervous system. Barret et al.[28] (French Perinatal Cohort Study) reported that the majority of established and possible cases of mitochondrial disorders had evidence of developmental delay. No other large cohorts have reported on this outcome among HIV-uninfected children although some smaller studies that examined this question have not found an association between developmental delay and ART exposure.[38,41]

8. Febrile Seizures

Febrile seizures are uncommon in children <9 months or >5 years of age, but approximately 3–4% of children between 1 and 4 years of age will experience a febrile seizure.[42] Febrile seizures are also a non-specific symptom of mitochondrial disorders and were noted in four of eight original cases reported by Blanche et al.[4] Landreau-Mascaro et al.[43] (French Perinatal Cohort Study) reported no difference in the incidence of neonatal seizures (seizures in infants <28 days old) between children exposed to ART (16 of 2635; 0.6%) and those who were not exposed to ART (9 of 1736; 0.5%). In contrast, children exposed to ART (primarily zidovudine or zidovudine plus lamivudine) were 2.7 times more likely to experience at least one febrile seizure (isolated seizure with fever at age >3 months) before the age of 18 months (24 of 2635 [1.1%] among ART-exposed infants vs 6 of 1736 [0.4%] among unexposed infants; $p = 0.02$). Chotpitayasunondh et al.[41] (Bangkok Collaborative Perinatal HIV Transmission Study Group) also found that seizures before the age of 18 months were more common in children exposed to zidovudine *in utero* (5 of 171; 2.9%) than in those exposed to placebo (1 of 159; 0.6%), although the study was not powered for this endpoint ($p = 0.21$). Hankin and colleagues (European Collaborative Study, 2003)[35] identified three cases of febrile seizures among 1406 children (0.2%) who were not exposed to ART and none among 1008 exposed to ART (not statistically significant), but such a low overall rate of febrile seizure suggests under-reporting.

Overall, these findings suggest that exposure to ART (and zidovudine specifically) may be associated with an increased risk of febrile seizure in children between 3 and 18 months of age, although the incidence of seizure in ART-exposed and ART-unexposed children has not been estimated with precision.

9. Lactic Acidaemia and Hyperlactataemia

The studies of lactic acidaemia have been limited to ART-exposed children. Among 38 HIV-uninfected children, Alimenti et al.[44] reported two cases of symptomatic lactic acidaemia (>5 mmol/L). Both presented at 2 weeks of age with recurrent vomiting and irritability. Both were taking oral zidovudine for prophylaxis at the time. The first was exposed *in utero* to zidovudine, lamivudine and nelfinavir as well as heroin, cocaine and methadone for 17 weeks. The second was exposed *in utero* to didanosine, stavudine and indinavir for 37 weeks with no reported exposure to illicit drugs. Lactate levels and symptoms resolved in both infants within 2 weeks of discontinuing oral zidovudine. In addition to the symptomatic cases, eight asymptomatic cases of serious elevations in serum lactate levels (>5 mmol/L) were also identified. Overall, 92% (35 of 38) of children had at least one abnormally high serum lactate measurement (≥2.1 mmol/L).

Noguera et al.[45] studied 127 HIV-uninfected, ART-exposed infants. Lactate levels were elevated on at least one occasion in 50% (n = 63) of infants with 30% (19 of 63) still showing elevated lactate levels at 1 year of age. Three infants had neurological symptoms consistent with mitochondrial disorders and concurrent hyperlactataemia. The first (exposed to didanosine, stavudine and nelfinavir *in utero* and zidovudine neonatally) presented at 2 months with weak head control, axial hypotonia and required 5 months of early stimulation to encourage gross motor development. The second (exposed to zidovudine, lamivudine, nevirapine and benzodiazepines *in utero* and zidovudine neonatally) presented at 2 weeks with weak head control, limb spasticity and preservation of primitive reflexes. The third (exposed to lamivudine, stavudine, nevirapine and tobacco *in utero* and zidovudine neonatally) presented at 6 months with weak head control, upper limb spasticity and inability to sit independently. All three experienced normalisation of symptoms and lactate levels by 1 year of age. None of the other infants had symptoms of lactic acidaemia.

Giaquinto et al.[46] reported that 17 of 20 (85%) ART-exposed children had at least one lactic acid level exceeding 2.5 mmol/L; however, all levels returned to normal during follow-up and none of the children developed any clinical symptoms consistent with mitochondrial disorders.

10. Neutrophils, Lymphocytes and Platelets

Le Chenadec et al.[47] (French Perinatal Cohort Study) prospectively measured neutrophils, platelets, CD4+ lymphocytes and CD8+ lymphocytes in 4249 uninfected infants through 18 months of age. Two-thirds of infants (2745 of 4249) were exposed to zidovudine monotherapy or regimens that included zidovudine. The vast majority (92%) were exposed both *in utero* and postnatally. Unexpectedly, the authors found decreased levels of neutrophils, lymphocytes and platelets that persisted through 18 months of age in multivariable models adjusted for infant's sex, mother's geographic origin, maternal drug use during pregnancy and prematurity. Compared with unexposed infants, absolute neutrophil counts were 234 and 287 $\times 10^6$ cells/L lower at 18 months in infants exposed to zidovudine monotherapy and zidovudine combination therapy, respectively. Lymphocyte counts were 200 and 251 $\times 10^6$ cells/L lower and platelet counts were 10 and 16 $\times 10^9$ cells/L lower at 18 months in infants exposed to zidovudine monotherapy and zidovudine combination therapy, respectively.

Bunders and colleagues (European Collaborative Study, 2004),[48] also found deficits in neutrophils that persisted through 8 years of age with an average difference in z-score of −0.15 (p = 0.04) after adjusting for child's sex, ethnicity, prematurity, maternal injection drug use and year of birth. Lambert et al.[49] reported that 5 of 39 uninfected infants exposed to ART pre- and postnatally developed neutropenia (severe in two) and 2 of 39 had platelet counts of <100 000. All had resolved at the time of study completion, but details of the exact timing were not given.

The authors suggest that the observed persistent changes in multiple cell lines may reflect toxic ef-

fects on multi-potent stem cells, consistent with findings from *in vitro* studies.[50,51] The clinical consequences of these deficits appear to be minimal in children <8 years of age, but it is not yet known if longer term effects will manifest. The fact that these effects persisted after discontinuation of ART is a source of concern that warrants additional investigation.

11. Anaemia

Le Chenadec et al.[47] (French Perinatal Cohort Study) found transient decreases in haemoglobin in 2745 ART-exposed infants that normalised by 3 months of age, consistent with findings from the ACTG (AIDS Clinical Trials Group) 076 randomised trial.[1] Hankin and colleagues (European Collaborative Study, 2003)[35] obtained data regarding haemoglobin levels within the first 6 weeks of life in 763 of 2414 uninfected infants. Of those, 458 were exposed to ART and 305 were unexposed to ART. Mean haemoglobin level was 12.6 g/dL among infants exposed to ART compared with 13.8 g/dL among unexposed infants ($p = 0.05$). The risk of anaemia was 2.7 times higher among ART-exposed children (33% vs 12%; $p < 0.001$). Haemoglobin level returned to normal upon discontinuation of ART in all affected infants.

Among 46 Thai infants (two of whom were HIV-infected) exposed to ART *in utero* and during the first 4 weeks of life, Panburana et al.[52] found that 26% had anaemia at birth (haemoglobin level <14 mg/dL) and 65% at 1 month (<10 mg/dL). None required transfusion and all cases of anaemia resolved by 2 months of age. Lambert et al.[49] reported that 27 of 39 uninfected infants exposed to ART pre- and postnatally had mild or moderate anaemia. All had resolved at the time of study completion, but details of the exact timing were not given. Among 126 uninfected infants exposed to combination therapy *in utero* and zidovudine postnatally, Bellón Cano et al.[40] reported that 29% had anaemia with one requiring transfusion.

Chotpitayasunondh et al.[41] (Bangkok Collaborative Perinatal HIV Transmission Study Group) reported that mean haematocrit level was lower at birth among 171 infants exposed *in utero* to zidovudine (49.1%) compared with 159 infants exposed to placebo (51.5%; $p < 0.001$). These infants were not exposed to zidovudine postnatally and no differences in haematocrit level were observed at 2, 6 and 18 months of age. Among all 395 children in the study (55 of whom were HIV-infected), a total of three cases of anaemia were identified at birth (2 of 196 ART-exposed and 1 of 199 ART-unexposed), all of which subsequently resolved.

Although transient anaemia among ART-exposed neonates appears to be quite common, the clinical significance of this finding is less clear. Abnormal haemoglobin levels typically normalise with discontinuation of ART. While anaemia may indicate mitochondrial dysfunction,[53] it is also a known adverse effect of zidovudine due to macrocytosis.[20] These studies have not reported other signs and symptoms of mitochondrial disorders in the children with anaemia. Studies with relatively small sample sizes are unlikely to be able to distinguish mitochondrial disorder-related anaemia from the high background rates of zidovudine-related anaemia.

12. mtDNA Depletion

Poirier et al.[54] (Women and Infants Transmission Study) found depletion of mtDNA in all ten uninfected children exposed to zidovudine. At 2 years of age, they had, on average, 32% lower mtDNA levels than ten children born to HIV-infected mothers who did not receive zidovudine (202.7 vs 297.3 copies of mitochondrial D loop gene per copy of 18S RNA gene; $p = 0.04$) and 58% lower mtDNA levels than 30 children born to HIV-uninfected women (202.7 vs 486.5 copies of mitochondrial D loop gene per copy of 18S RNA gene; $p < 0.05$).

Two additional studies have published relevant results, although it is not possible to distinguish between the effects of HIV and those of NRTIs. In results from a pilot study, Divi et al.[55] reported that five of nine infants exposed *in utero* to zidovudine plus lamivudine displayed moderate to severe mitochondrial morphological damage in endothelial cells

from umbilical cord tissue compared with zero of seven infants born to uninfected mothers. The degree of mitochondrial damage was significantly correlated with duration of antiretroviral exposure. HIV-exposed infants whose mothers received zidovudine plus lamivudine also had significant depletions of mtDNA in umbilical cord endothelial cells (323 vs 512; p = 0.006) and cord blood (339 vs 544; p = 0.003) compared with HIV- and NRTI-unexposed infants. All of the exposed children were clinically asymptomatic.

In an analysis of mtDNA conducted by Shiramizu et al.,[56] infants born to HIV-infected women on NRTI therapy (n = 8) had 83% fewer mtDNA copies/cell in placental tissue (mean 152 vs 880 copies/cell; p = 0.0016) and cord blood (mean 144 vs 865 copies/cell; p = 0.0026) than infants born to HIV-uninfected women (n = 5). These data suggest that HIV and/or ART exposure *in utero* causes depletion of mtDNA, but it is not known what if any clinical consequences result from these changes.

13. Discussion

This systematic review of the published literature found inconsistent reports regarding the most clinically relevant outcomes (morbidity and mortality) of mitochondrial dysfunction, while changes in laboratory values with unknown clinical significance were more consistently related to perinatal ART-exposure. To briefly highlight key results, the French Perinatal Cohort Study (n = 4072) reported significantly greater numbers of cases of 'established' and 'possible' mitochondrial disorders among ART-exposed infants than among ART-unexposed infants while follow-up of living, HIV-negative children in the Pediatric ACTG (n = 1960) trial found no cases of mitochondrial disorders among ART-exposed infants.

Results from studies of mortality are also conflicting with the French Perinatal Cohort Study reporting two deaths attributable to mitochondrial disorders (both among ART-exposed children) while the Perinatal Safety Review Working Group (n = 23 265) found three deaths 'possibly' related to mitochondrial disorders, none of which were in children likely to have been exposed to antiretrovirals.

The data suggest an increased risk of transient elevations in lactate levels, anaemia and mtDNA depletion in infants exposed to ART as well as longer term changes in lymphocytes, neutrophils and platelets. We conclude that large, prospective studies of HIV-exposed infants are needed to resolve the discrepant results regarding morbidity and mortality related to mitochondrial disorders, to ascertain the clinical significance of effects on laboratory values, to determine whether or not the incidence of mitochondrial disorders differs by regimen and to develop predictive models that might identify which infants are at the greatest risk. We will highlight several key challenges that remain to be addressed in the design and implementation of studies to answer these questions.

13.1 Large Cohorts in Resource-Limited Settings

Mitochondrial disorder is a rare outcome even with the elevated risk in HIV-exposed infants. Large numbers of HIV-exposed infants are needed to estimate the effect of ART exposure on mitochondrial disorders with precision and an additional order of magnitude increase in sample size would be needed to detect differences (if any) between ART regimens. Only three studies published to date have attempted to systematically review clinical data from a large enough population of children to detect the effect of ART on the risk of mitochondrial disorders.[28,35,36] These have all been conducted in resource-rich settings with the study population accrued over a period of many years.

The ethical principal of justice as well as logistical reasons demand that future studies be conducted in resource-poor settings where HIV and any adverse effects of ART for the prevention of mother-to-child transmission will have the greatest public health impact. Limited healthcare infrastructure in these resource-poor settings means that study design and implementation cannot rely on data collected in the routine course of clinical care. These studies will necessarily be prospective in nature to collect accu-

rate data on the timing and type of antiretroviral exposure during pregnancy as well as other risk factors for mitochondrial disorders. For this reason, case-control studies, which are generally more cost effective for rare outcomes, are probably not feasible.

The clinical significance of long-term changes in neutrophils, lymphocytes and platelets has not yet been identified. Likewise, developmental delays may be transient or lasting. And it is not known whether any of these effects of *in utero* ART exposure will be evident in children with suboptimal nutrition and healthcare. These important longer term outcomes will be challenging to study given the difficulty of on-going follow-up for uninfected children over a period of many years.

13.2 Screening for Mitochondrial Disorders

The definitive diagnostic test for mitochondrial disorders, muscle or organ biopsy, is highly invasive. Obtaining muscle biopsy from asymptomatic children is neither ethical nor feasible.[57] A sensitive and relatively inexpensive method of screening for mitochondrial disorders is needed for use in prospective cohort studies in resource-limited settings. The French Perinatal Cohort Study is a rich source of clinical and laboratory data combined with careful ascertainment of mitochondrial dysfunction. An initial screening algorithm might be developed based on this cohort using the subset of patient characteristics (clinical signs, symptoms and laboratory values) that can be obtained in resource-poor settings to facilitate large scale studies. Statistical modelling using all patient characteristics in the full dataset would be needed to assess the degree to which specific characteristics (or combinations thereof) are sensitive indicators of possible mitochondrial disorders, although the cases reported by Barret et al.[28] suggest that neurological symptoms (developmental delay, seizures) and/or persistent hyperlactataemia may be candidates. Further evaluation of such a screening algorithm would be needed to determine how well it performs outside of the French population.

Given the low prevalence of mitochondrial disorders, a sensitive screening test would be expected to identify many more false positives than true positives. Possible cases would need to be confirmed. Because of the invasive nature of muscle biopsy, a validated set of diagnostic criteria using less invasive methods would also be an invaluable tool in the ongoing effort to understand the potential for mitochondrial disorders in neonates who will necessarily be exposed to antiretroviral drugs to prevent vertical transmission of HIV infection.[58] The criteria used by the French Perinatal Cohort Study draw on the characteristics of children with constitutional mitochondrial disorders as well as the presentation of mitochondrial toxicity in NRTI-exposed adults, but the sensitivity and specificity of these diagnostic criteria have not yet been formally studied.

13.3 Unknown Background Rate of Mitochondrial Disorders in HIV-Exposed Infants

Barret et al.[28] (French Perinatal Cohort Study) estimated the cumulative incidence of mitochondrial disorders in ART-exposed infants at 26 cases per 10 000 children up to the age of 18 months. While this is dramatically higher than the reported prevalence of mitochondrial disorders in the general paediatric population of 0.3 case per 10 000 to 1 case per 10 000,[8-11] caution should be exercised in comparing the background rate of mitochondrial disorders in ART-unexposed, uninfected infants born to HIV-infected mothers with that of the general paediatric population. Many of the known risk factors for mitochondrial disorders are more common among HIV-infected persons in the populations studied to date including alcohol use, concurrent medications and hepatitis co-infection (table II). There are also data to suggest that HIV itself can cause mitochondrial dysfunction, which could lead to elevated rates of mitochondrial disorders among infants born to HIV-infected women compared with the general population.[54] The best available estimate of the incidence of mitochondrial disorders among ART-unexposed infants born to HIV-infected women comes from the French Perinatal Cohort

Table II. Risk factors for mitochondrial disorders[30,42,59]

Alcohol
Endogenous bile acids
Female sex hormones
Hepatitis B or C infection
Drugs (especially long-term exposure)
amineptine, tianeptine
amiodarone
anthracyclines
aspirin (acetylsalicylic acid)
chloramphenicol
diethylaminoethoxyhexestrol
hydroxycarbamide
interferon-α
iron
NSAIDs
perhexiline
tetracycline
valproic acid
Nutritional factors
vitamin B_{12} excess
vitamin B_{12} deficiency
iron excess
Inherited and sporadic mitochondrial DNA and nuclear DNA mutations
mitochondrial encephalomyopathy, encephalomyopathy, lactic acidosis and strokelike episodes (MELAS)
myoclonus epilepsy and ragged-red fibers (MERRF)
Leber hereditary optic neuropathy (LHON)
ATPase subunit 6 mutation (NARP)
Leigh syndrome (subacute necrotising encephalomyopathy)

Study (n = 1748), which found no cases of mitochondrial disorders in these infants for a one-sided, upper 97.5% confidence limit of 21 cases per 10 000. Given the imprecise nature of this estimate, it is unclear whether or not the background rate of mitochondrial disorders is greater in infants born to HIV-infected women than HIV-uninfected women.

Ideally, observational cohort studies of mitochondrial disorders in HIV-exposed children would include those unexposed to ART to enable investigators to tease apart the effects of ART from those of HIV itself. While <10% of pregnant HIV-infected women worldwide receive ART for the prevention of mother-to-child transmission,[60] perinatal prophylaxis is known to be effective and one could not ethically randomise pregnant women to placebo versus ART nor invest heavily in an observational study without offering prophylaxis to the participants (studies published to date have primarily included unexposed pregnancies from pre-1994, the era before the results of ACTG 076 were reported). Because of these ethical considerations, it may be more feasible to study differences between regimens despite the fact that larger numbers of participants will be needed to detect those differences.

13.4 Disentangling Components of Regimens

Given the unarguable importance of preventing vertical transmission of the virus, the critical question for current research is to determine which ART regimens have the lowest risk of mitochondrial disorders for the infant exposed *in utero*. Although not yet published at the time of this review, results from 1037 children enrolled in the Pediatric ACTG 219 and 219C studies (presented in Toronto at the XVI International AIDS Conference, August 2006) indicate that exposure late in pregnancy to lamivudine with or without zidovudine may be associated with an elevated risk of 'possible' mitochondrial disorders (OR for lamivudine = 10.06; 95% CI 1.83, 72.45; OR for lamivudine plus zidovudine = 9.46; 95% CI 1.70, 69.30), but this is based on a small number of exposed cases (n = 6).[61] We identified no published studies that were adequately powered to assess the variation in risk of mitochondrial disorders in infants and children by regimen. Noguera et al.[45] examined the relationship between specific drugs and lactate levels. The only exposure significantly associated with hyperlactataemia was gestational use of didanosine (OR = 1.06 per additional week of fetal exposure; 95% CI 1.01, 1.11). A few studies have looked broadly at adverse events associated with different prophylaxis regimens. Taha et al.[62] randomly assigned 894 infants born to HIV-infected mothers in Malawi to receive either nevirapine alone or nevirapine plus zidovudine for 1 week after birth. The rates of grade 3 or 4 adverse events were not statistically different in the two groups (nevirapine = 4.9%; nevirapine plus zidovudine = 5.4%; p = 0.76). There was an indication of greater risk of severe haematological changes in those re-

ceiving nevirapine plus zidovudine (4.4%) versus nevirapine-only (2.1%), but the study was not powered to estimate this difference with precision. Bellón Cano et al.[40] compared adverse events in infants exposed to combination therapy with two NRTIs (with or without a non-NRTI) versus two NRTIs plus a PI. They reported similar proportions of children with anaemia in both groups. Although identifying the separate effects of individual drugs would be particularly challenging in the developed world where combinations that include three or more drugs are now the norm, this issue may be more tractable in less-developed settings where monotherapy and dual therapy are commonly used.

13.5 Identifying at Risk Infants

Few published studies have examined risk factors for mitochondrial disorders in HIV-exposed infants and children apart from the role of ART. Earlier maternal initiation of therapy has been linked to degree of mitochondrial damage,[55] and the duration of *in utero* exposure to didanosine (but not ART overall or other specific drugs) was associated with an increased risk of elevated lactate levels.[45] Divi et al.[55] found no significant correlation between the levels of mtDNA depletion and maternal CD4 percentage, CD4 cell count, viral load or age. Noguera et al.[45] also found no other predictors of hyperlactataemia among all the clinical and laboratory values that they examined. The large cohort study that we propose should have the necessary data and sample size to develop predictive models that might help to identify infants at greater risk of mitochondrial disorders.

14. Conclusion

Antiretrovirals are essential for the prevention of mother-to-child transmission of HIV. Despite a potential small excess risk of mitochondrial disorders with exposure, the benefit to infants clearly justifies the use of ART prophylaxis. We conclude that a large international cohort of HIV-exposed infants is needed to better characterise the relationship between ART exposure and mitochondrial disorders. The challenges that remain to be addressed include development of a sensitive but affordable screening algorithm in combination with specific diagnostic criteria; consistent collection of data on ART exposure and other risk factors, long-term follow-up of HIV-exposed children and implementation in resource limited settings. With these data in hand, policy makers and healthcare providers could make informed decisions about the choice of antiretrovirals for prevention of mother-to-child transmission that would minimise the risk of mitochondrial disorders while maximising the public health benefit of these drugs.

Acknowledgements

This work was supported by GlaxoSmithKline (GSK) Research & Development. We would like to thank the anonymous peer reviewers who provided thoughtful and valuable suggestions.

Dr Jonsson Funk conducted an early version of this review as a paid consultant for GSK. Dr Belinson was employed on a part-time basis by GSK while undertaking this work. Dr Morsheimer worked on this review as a paid consultant for GSK. Dr Pimenta and Dr Gibbons are employed full time by GSK.

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