AIDS RESEARCH AND HUMAN RETROVIRUSES
Volume 26, Number 9, 2010

DOI: 10.1089/aid.2009.0273

Evolution of Mitochondrial DNA Content After Planned Interruption of HAART in HIV-Infected Pediatric Patients

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Abstract

HAART-related long-term toxicities, many of them ascribed to mitochondrial (mt) toxicity of the nucleoside analogues, are being increasingly reported in HIV-infected children. HIV infection can also cause mt damage. Case series include 13 vertically HIV-infected pediatric patients (9 girls, median age 10.5 years) with optimal long-term response to a first-line HAART regimen who underwent planned treatment interruption (PTI). MtDNA content from peripheral blood mononuclear cells was assessed by means of a real-time PCR technique at PTI and 12 months later and expressed as an mtDNA/nuclear DNA ratio, together with lactate levels. At PTI, patients had remained a median time of 4.7 years on HAART and 4.3 years with complete suppression of viral replication. The main reason leading to PTI was treatment fatigue. One month after PTI, HIV plasmatic viral load had increased to 4.8 log copies/ml and stabilized thereafter. During the 12-month study period, all children remained free from any HIV-related clinical event. A progressive and significant decrease in median CD4 cell counts and percentages was observed 12 months after PTI. One year after PTI, the median mtDNA/nuclear DNA ratios had increased from 0.76 to 1.08 ($p = 0.002$) and lactate levels had decreased (from 1.12 to 0.73 mmol/liter; $p = 0.019$). Changes in mtDNA did not correlate with changes in lactate levels. No relationship was found between the evolution in mt toxicity markers and the rest of the clinical, immunological, and virological variables. In this series, PTI led to a partial restoration of mtDNA levels and a significant decrease in lactate values.

The implementation of highly active antiretroviral (ARV) therapy (HAART) has significantly improved morbidity and mortality from HIV infection/AIDS in the pediatric population.[1] Nucleoside analogue reverse transcriptase inhibitors (NRTIs) remain the backbone of most HAART regimens, especially among children, for whom many of the newest drugs have not yet been licensed. NRTIs also inhibit DNA polymerase gamma, which can lead to mitochondrial (mt) DNA (mtDNA) depletion, and to mt dysfunction. HAART-related long-term toxicities are being increasingly reported in vertically HIV-infected pediatric patients.[2,3] Many of these have been ascribed to direct mt toxicity of the NRTIs. Further investigation on the pathogenesis, clinical manifestations, and therapeutics of these toxicities in children is mandatory, as these patients shall be exposed to ARV for an ever-increasing length of time throughout postnatal growth and development. We report our experience with HAART interruption in a series of HIV-infected children and its effects on biological markers of mt toxicity.

This was a prospective case series of perinatally HIV-infected pediatric patients followed up in a third-level pediatric hospital in Barcelona (Spain) who underwent planned treatment interruption (PTI). At the time of PTI, all patients fulfilled the following requirements: to be on a first-line HAART, freedom from any active HIV-related clinical condition, undetectable plasmatic HIV-RNA (limit of <50 copies/ml; CA HIV Monitor; Roche, Basel, Switzerland), and a maintained immune situation (flow cytometry, FACSCalibur; BD Biosciences, San Jose, CA) within CDC Category 1 (>350 cells/mm^3 for adolescents or >25% for children aged 12 years or less) for at least the past 2 years. ARV treatment

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reinitiation was planned in case any HIV-related laboratory (grade 3 or 4 anemia, thrombocytopenia, leukopenia, or elevation of liver enzymes according to the Division of Acquired Immunodeficiency Syndrome toxicity grades[4]) or clinical event, or a marked decrease in CD4 cells (<350 cells/mm^3 for adolescents or <20% for children aged 12 years or less) was observed. Informed consent was obtained from parents or legal guardians. The study was approved by the Ethics Committee of the Hospital Sant Joan de Déu. There was a close clinical and biological follow-up after PTI was performed, with monthly controls during the first 6 months and every 2–3 months thereafter.

Peripheral blood mononuclear cell (PBMC) samples were obtained by standard methods (density gradient centrifugation in a dextran medium) and cryopreserved at −80°C. MtDNA content from cryopreserved PBMCs was assessed at the time of PTI (baseline) and 12 months later. Plasmatic lactate levels (normal values: 0.55–1.77 mmol/liter) were also obtained at these two time points; given the physiological variability and lack of specificity when determining lactate concentrations, previously reported strict validation criteria were used.[5] DNA extraction from PBMCs was performed by the standard phenol-chloroform isolation protocol.

Total DNA was quantified by spectrophotometry and 2 ng of each sample was amplified by real-time polymerase chain reaction (rtPCR) using LightCycler technology and SYBR Green I dye (Roche, Indianapolis, IN). DNA quantification by PCR is based on the ability of fluorescent SYBR Green I to bind double-stranded DNA. Both DNA and SYBR Green fluorescence duplicate exponentially in each PCR cycle. Fluorescence intensity can be measured all along the amplification process and is proportional to the final DNA amount and, therefore, to the initial DNA concentration. To assess mtDNA content the rtPCR was carried out separately to amplify a sequence of a highly conserved mt ND2 gene and a fragment of the nuclear-coded housekeeping 18S rRNA gene. MtDNA content was expressed as the ratio of ND2 mtDNA with respect 18S rRNA nDNA.

The one-sample Kolmogorov–Smirnov test was used to assess that continuous data followed a normal distribution. Changes for mtDNA, lactate, CD4 cell percentage, and viral load were evaluated by means of a paired *t*-test. Statistical significance was set at 0.05.

From June 2001 to June 2006, 13 patients underwent PTI and completed a 12-month period off therapy (9 girls, median age at PTI: 10.5 years; age range: 3.9–16.0 years; Table 1). At the time of PTI, patients had remained on HAART a median time of 4.7 years and 4.3 years with complete suppression of viral replication. The main reason leading to PTI was treatment fatigue.

During the 12-month study period, all children remained free from any HIV-related clinical event. As expected, an increase in RNA-HIV plasmatic load up to a median value of 4.8 log copies/ml (range 2.9–5.6; $p < 0.0001$) was observed in all cases 1 month after PTI; HIV viremia stabilized thereafter (median values 12 months after PTI: 4.5 log, range: 3.2–5.1; $p = 0.32$ when compared to the initial blip 1 month after PTI). A progressive and significant decrease in median CD4 cell counts and percentages was observed 12 months after PTI (from 858 to 504 cells/mm^3, $p = 0.002$; and from 36.0% to 28.0%, $p < 0.0001$), with the following slopes of decline: −30 CD4 cells/mm^3 per month and −0.8% per month.

Baseline median ND2 mtDNA/18S rRNA nDNA ratios and lactate levels were as follows: 0.76 (range: 0.09–1.79) and 1.12 mmol/liter (range: 0.67–3.00 mmol/liter); no differences in mtDNA ratios or lactate levels were observed between children who received didanosine (ddI, $n = 8$) or stavudine (d4T, $n = 10$) or ddI plus d4T ($n = 7$) and those who did not at the time of PTI. No other association was observed between mt toxicity markers at baseline and background characteristics of the patients (sex, age, CDC clinical category, duration of therapy, nadir CD4 cell count or percentage, highest plasma HIV-RNA value, and HAART regimen).

One year after PTI, ND2 mtDNA/18S rRNA nDNA ratios had increased in seven patients and had decreased in six patients, with variations ranging from −0.59 to 2.57 (paired

Table 1. Baseline Clinical and Immunological Characteristics of Patients Undergoing Planned Interruption of HAART[a]

Gender and CDC clinical category			*Nadir CD4 cells*		*At PTI*			*12-month evolution*	
			/mm^3	*%*	*HAART regimen*	*Main reason*	*Age (years)*	*Δ LA*	*Δ mtDNA*
1	M	A	882	21	d4T-ddI-NFV	tx fatigue	10.5	−0.5	2.57
2	F	B	792	22	d4T-ddI-NFV	Hepatic toxicity	8.4	−1.34	1.21
3	F	A	1127	23	d4T-ddI-NFV → NVP	tx fatigue	12	−0.73	0.77
4	F	C	300	21	d4T-NVP-NFV	tx fatigue	13.2	−0.04	1.69
5	M	A	736	23	ddI-d4T-NFV → NVP	Psychiatric tx	14.3	−0.45	−0.36
6	F	C	2496	32	ABC-ddI-d4T-NFV	tx fatigue	5.5	−0.42	1.45
7	F	B	310	21	d4T-3TC-EFV	Central obesity	12.7	−0.16	−0.38
8	F	A	882	18	ZDV-3TC-RTV → NVP	tx fatigue	7	−0.28	−0.59
9	M	B	756	26	d4T-ddI-NFV	tx fatigue	12.6	−0.21	0.77
10	M	B	442	13	3TC-ZDV-NVP	Facial lipoatrophy	16	−0.51	0.85
11	F	A	226	14	d4T-ddI-NVP-NFV	tx fatigue	4.9	−0.2	−0.26
12	F	A	1100	25	d4T-3TC-NFV	tx fatigue	3.9	0.41	−0.01
13	F	A	1200	31	AZT-ddI-NVP	tx fatigue	4.1	−1.94	−0.06

[a]M, male; F, female; PTI, planned treatment interruption; d4T, stavudine; ddI, didanosine; NFV, nelfinavir; NVP, nevirapine; ABC, abacavir; 3TC, lamivudine; EFV, efavirenz; ZDV, zidovudine; RTV, ritonavir; tx, treatment; LA, lactate in mmol/liter; mtDNA, mitochondrial DNA.

t-test, $p = 0.002$; Fig. 1a); overall, a median net increase up to 1.08 (range: 0.08–4.00) was observed. A significant decrease in median lactate levels was also noted (median value 0.73 mmol/liter, range: 0.31–1.62; paired *t*-test, $p = 0.019$); lactatemia decreased in all but one patient, with changes ranging from −1.94 to 0.41 mmol/liter (Fig. 1b). Changes in ND2 mtDNA/18S rRNA nDNA ratios did not correlate with changes in lactate levels. Again, no relationship was found between the evolution in mt toxicity markers and the rest of the clinical, immunological, and virological variables (data not shown).

After the SMART trial,[6] treatment interruptions in the adult patient are strongly discouraged. The natural history of vertically transmitted HIV infection is different from that of the adult and, therefore, response to PTIs in children may as well be different. To pose minimal risk to patients, only children fulfilling very stringent requirements were selected to undergo PTI in our study (i.e., age over 2 years, when the risk of progression to AIDS diminishes, to be on a first-line HAART, and long-term normal CD4 cell counts and complete suppression of viral replication). The clinical, immunological, and virological evolution we observe is very similar to that reported by the only clinical trial on PTIs in HIV-infected children published to date[7] and warrants the need to further investigate this therapeutic strategy in this population.

To date, few studies have focused on mt function markers in HIV-infected children. In a 2-year follow-up including 80 HIV-infected pediatric patients, we reported the calculated incidence of asymptomatic hyperlactatemia to be 8.7 per 100 patient-years; a younger age at the beginning of HAART was the only risk factor for developing hyperlactatemia by logistic regression analysis.[5] Similar figures have been observed by other authors, both in children[8] and adult patients.[9] ARV-related mt dysfunction in the pediatric age group is rarely symptomatic. In addition to some cases of lactic acidosis,[10,11] mt toxicity in children usually manifests with neurological symptoms. Of note, ARV-related mt toxicity also affects HIV-uninfected infants who were exposed perinatally to ARV, in whom neurological syndromes similar to those classically described in inherited mt diseases have been reported.[12,13]

Only three small studies have investigated mtDNA levels in the PBMCs of HIV-infected children. Saitoh *et al.*,[14] in a 104-week follow-up of 31 patients on stable HAART and suppression of viral replication, identified ddI as the only NRTI associated with mtDNA suppression in PBMCs before and during HAART. Another study showed no difference in mt function or content between HIV-infected children with ($n = 6$) or without lipodystropy ($n = 12$).[15]

Recently, the metabolic and mt function evolution was investigated in a group of 18 patients who were randomized either to continue on a d4T-containing HAART regimen (arm A, $n = 9$) or to switch to tenofovir (arm B, $n = 9$); although a significant decrease was observed in plasma HDL-cholesterol in arm B, no other changes were noted in metabolic parameters or mtDNA levels after a 18-month follow-up.[16] To our knowledge, the effects of HAART interruption on mt function in children have not been investigated to date. In our small series, a 12-month treatment interruption led to a partial restoration of mtDNA levels in PBMCs and to a decrease in plasmatic lactatemia. Whether these changes leads to clinical improvements in the long term remains uncertain. Likewise, it is unknown if similar changes in mtDNA also occurred in other tissues known to be affected by clinically relevant ARV-related mt toxicity, such as fat.[17]

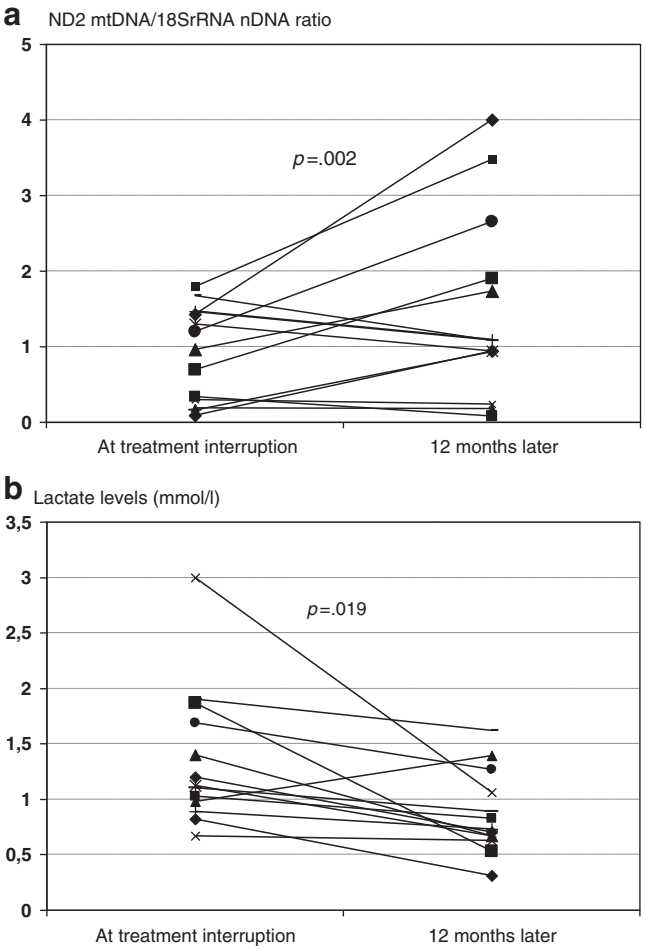

FIG. 1. Twelve-month evolution in ND2 mtDNA/18S rRNA nDNA ratios (**a**) and lactate levels (**b**) in the 13 patients undergoing planned HAART interruption.

The deleterious effect of HIV infection on mtDNA content was demonstrated early by Côté and colleagues,[18] as was its partial restoration after treatment interruption.[18,19] We have also reported a decrease both in mtDNA content and in mt respiratory chain enzyme activities of complexes partially encoded by mtDNA in HIV-infected adult patients who were never treated with ARV when compared with healthy controls.[20] Other authors have even described an inverse correlation between HIV plasmatic viral load and mtDNA content in PBMCs.[21] In our study, despite a global and significant increase in mtDNA levels, these decreased in almost half of the patients following HIV viremia rebound after PTI, although these decreases were of smaller magnitude. Our results suggest that mtDNA loss in HIV-infected children is both due to the use of NRTIs and to the deleterious effects of HIV on mitochondria, although persistent mt damage cannot be discarded.

We were not able to show the association between ddI or d4T and mt toxicity reported by other authors,[22] probably because of the small numbers and because most of the patients were receiving either of those drugs or both at the time of treatment interruption. Moreover, our preliminary results

should be taken with caution as we could not completely decontaminate blood samples of platelets, which contain mtDNA but not nuclear DNA[23]; proper elimination of platelets after density gradient centrifugation requires large volumes of whole blood, and these are often not available in children. Considering the growing number of children who will be exposed to these agents in the following years, in our opinion, continuous investigation of ARV-related toxicity in the pediatric age is warranted.

Acknowledgments

Fundació la Marató de TV3 (020210 and 020631), FIPSE 36612/06, FIS 99/0051-01, 40381/04, and 41239/04, Suports a Grups de Recerca de la Generalitat de Catalunya (2005/SGR/0300), and CIBER de Enfermedades Raras (initiative of the Instituto de Salud Carlos, ISCIII) are acknowledged for support. Núria Rovira was the recipient of a grant from Hospital Sant Joan de Déu. Òscar Miró was the recipient of a grant for Research Intensification from ISCIII in 2009.

Author Disclosure Statement

No competing financial interests exist.

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