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Rapid decline of HIV-1 DNA and RNA in infants starting very early ART may pose a diagnostic challenge

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Abstract

Objective—Birth diagnosis of HIV-1 infection offers an ideal opportunity for early antiretroviral treatment (ART) to limit HIV-1 reservoir size and limit disease progression. Although data on cellular HIV-1 DNA decay exist for children commencing treatment from 2–3 months of age, data is lacking for starting shortly after birth.

Design—We studied infants who initiated ART within 8 days after birth to assess HIV-1 DNA levels longitudinally.

Methods—Children were recruited from public health clinics in Cape Town where birth diagnosis of HIV-1 coupled with early ART initiation occurred. Total cellular HIV-1 DNA levels were determined using a sensitive quantitative PCR targeting a conserved region in *integrase*.

Results—Of 11 infants diagnosed and beginning ART within 8 days of birth with detectable pre-ART HIV-1 DNA, 3 subsequently had undetectable HIV-1 DNA after 6 days, 3 months and 4 months on treatment, respectively. In 7 who had virologic suppression (defined as a continuous downward trend in plasma HIV-1 RNA, and <100 copies/mL after 6 months) total HIV-1 DNA continued to decay over 12 months (mean half-life of 64.8 days [95% CI: 47.9–105.7]).

Conclusion—In infants initiated on ART within 8 days of life the combination of maternal ART, and early ART for prophylaxis and treatment contribute to rapid decline of HIV-1 infected cells to low or undetectable levels. However, rapid decline of HIV-1 RNA and DNA may complicate definitive diagnosis when confirmatory testing is delayed.

Introduction

Most intra-uterine HIV-1 infections occur during the last weeks of gestation[1]. Infant diagnosis by sensitive HIV-1 nucleic acid testing at birth offers a unique opportunity to

diagnose infection as soon as possible to begin therapy and linkage to care as infant HIV-1 disease is rapidly progressive with high mortality[2–5]. Early antiretroviral therapy (ART) can also limit the HIV-1 reservoir size[6–9]. Low reservoir sizes are associated with a delayed rebound after ART discontinuation, most likely due to stochastic activation of rare infected cells containing intact proviruses[10]. This was evident from a prolonged period without rebound viremia, despite absent detectable immune response in the Mississippi child and adult Boston hematopoietic stem cell transplant patients[11–14]. Early therapy may also provide an opportunity to achieve ART-free remission due to a small reservoir size and intact immune system: in adults, early therapy followed by interruption resulted in post treatment control in about 15% in the Visconti cohort[15], but data from the SPARTAC study suggest that the effect of early treatment may have been inflated by the natural occurrence of transient control early after infection[16,17]. Nevertheless, the proportion remains higher than naturally occurring elite controllers (<1%). Post treatment control was also observed in perinatally infected individuals: in two children beginning ART within the first 3 months of life[18,19] and a young adult who started therapy, after perinatal infection, at 3.5 years of age[20].

In children who initiated ART between 0.5 to 2.6 months of age a study described that HIV-1 DNA concentration decayed to 1.0 to 1.5 log₁₀ copies/million cells at 1–2 years of age[21]. Two other studies described median HIV-1 DNA half-lives of 53-[22] and 107 days[23], in children initiating ART around a median of 2 months or before 3 months, respectively. We have previously shown that therapy before 2 months of age reduces the number of infected cells and their transcriptional activity measured by unspliced cellular RNA[24]. However, information on the early decay of HIV-1 DNA in infants who began ART shortly after birth is limited. Our aim was therefore to investigate changes in total HIV-1 DNA in infants starting ART within 8 days after birth.

Methods

Children were diagnosed through a public health sector birth HIV-1 diagnosis program in Cape Town, South Africa, and initiated ART as soon as feasible. Parents or legal guardians provided informed consent. The study was approved by the Stellenbosch University's Health Research Ethics Committee (reference: M14/07/029).

HIV-1 infection was confirmed with at least 2 positive HIV nucleic acid tests on separate samples (qualitative and/or quantitative) with Roche COBAS[®] AmpliPrep/COBAS[®] TaqMan[®] (CAP/CTM) HIV-1 v2.0 or HIV-1 Qualitative v2 (CAP/CTM) (Roche Molecular Diagnostics, Pleasanton, CA). Subsequently the infants enrolled in a study of HIV-1 reservoirs and neurodevelopment in infants and children. We studied total cell associated HIV-1 DNA kinetics in infants beginning ART within 8 days of birth. Other inclusion criteria were having detectable baseline HIV-1 DNA and at least 2 stored peripheral blood mononuclear cell (PBMC) samples on treatment.

PBMCs and plasma were processed at 3 monthly visits. Samples were processed and stored according to the HANC Cross-Network PBMC processing SOP (<https://www.hanc.info/labs/labresources/procedures/Pages/pbmcSop.aspx>). HIV-1 total DNA was extracted and

measured through a sensitive quantitative PCR adapted for HIV-1 subtype C, targeting a conserved region in HIV-1 integrase (iCAD; limit of detection: 3 copies/million PBMCs; Supplementary Table 1)[25,26]. HIV-1 RNA was quantified with the CAP/CTM v2.0, with a 100 copies/mL limit of detection for a 200 microliter plasma input. We defined virologic suppression as a continuous downward trend in plasma HIV-1 RNA and no HIV-1 RNA > 100 copies/mL at the first measurement after 6 months on ART. Infants not meeting these criteria were classified as viremic.

As pre-treatment PBMCs were unavailable, we assayed pre-treatment dried blood spots (DBS), routinely stored in the diagnostic laboratory, to confirm assay detectability. The PBMC extraction method was adapted for DBS nucleic acid extraction: A solution comprising proteinase K and guanidinium hydrochloride was added to the dried blood spot, sonicated immediately for 10 seconds, incubated at 37°C overnight and followed by extraction and total HIV-1 DNA quantification as described[26].

Statistics: Kinetics were modelled with log-linear regression. To predict total HIV-1 DNA (log copies/mL), mixed effect models (participant as random effect and time-treated as fixed effect) were compared and random intercept models were found to be optimal (adding random slopes did not improve Akaike information criterion (AIC) or Bayesian information criterion (BIC)). Goodness of fit was assessed with a conditional generalized linear least square method and confidence intervals were calculated with bootstrapping[27,28]. Graphics and statistics were performed with R version 3.3.1[29]. We expressed decay rates as half-lives ($t_{1/2}$) which allows for an intuitive comparison between different phases of decay and with published studies.

Results

Fifteen infants started ART between 0 and 8 days (median 4 days) after birth (Table 1) and had at least 3 samples collected (a pre-ART DBS and at least two on-treatment PBMC samples). After enrollment, two infants had no detectable plasma HIV-1 RNA and cellular HIV-1 DNA samples, and another two had detectable plasma HIV-1 RNA only. The remaining 11 had at least one baseline assay-detectable HIV-1 DNA sample (pre-ART samples were from DBS) to allow longitudinal HIV-1 DNA observation, 7 of these were suppressed “S” and 4 viremic “V” participants (Figure; Supplementary Table 2).

Plasma HIV-1 RNA decay kinetics: Average baseline HIV-1 RNA was 3.9 (range 2.6–4.7) log 10 copies/mL in the 11 infants. In the 7 suppressed infants, HIV-1 RNA was undetectable (< 100 copies/mL for 200 microliter input) in 3 children within 3.4 months, and in one child it was 120 copies at 3.5 months. In the remaining 3 patients, the first undetectable plasma HIV-1 RNA loads were recorded between 6 and 7 months.

Cellular HIV-1 DNA decay kinetics: HIV-1 DNA decay slopes were characterized in the 7 suppressed infants. As pre-ART DBS samples contained cells other than PBMCs we used it only to assess that HIV-1 DNA could be detected by our assay but could not to characterize the decay between pre-ART and the first on-ART time point. Thereafter HIV-1 DNA time slopes showed a good log-linear correlation (mean $t_{1/2}$ (95% CI): 64.8 (47.9–105.7) days;

conditional R^2 (95% CI): 0.77(0.53–0.91); Figure). At the end of the observation period at a median of 6.9 months (95% CI 3.5–11.6 months), in 6 of 7 suppressed infants, HIV-1 DNA loads dropped to < 10 copies/million PBMCs. In one of these 6 HIV-1 DNA was undetectable at the second visit, 3.5 months on ART (participant S9) in another HIV-1 DNA remained detectable, but unquantifiable at < 3 copies/million PMBCs, at 11.6 months (participant S3). The “suppressed” child whose HIV-1 DNA remained > 10 copies/million PBMCs (participant S6), had delayed viremia suppression (145 HIV-1 RNA copies at 4 months on treatment) with HIV-1 DNA load reaching 47.8 HIV-1 DNA copies/million PBMCs at 6 months. Two “viremic” infants had early undetectable HIV-1 DNA at their first visits, respectively, 6 days (participant V5) and 4 months (participant V3) with subsequent viremia; participant V5 had continued viremia with accompanied HIV-1 DNA increase whereas participant V3 had a single episode of viremia with HIV-1 DNA remaining undetectable.

Discussion

In infants with a rapid plasma HIV-1 RNA load suppression, here defined as HIV-1 RNA < 100 copies/mL within 3.5 months, who had assay detectable HIV-1 DNA, total DNA became undetectable in 3 of 11 infants, respectively at 6 days, 3.5 months and 4 months on treatment. The rate of initial HIV-1 DNA decay from treatment initiation to study enrolment could not be accurately assessed since only DBS were available pre-treatment. Thereafter total HIV-1 DNA in PBMC cells decayed with a $t_{1/2}$ of 65 days during the remainder of the first year of life with all but one of seven suppressed infants reaching a total HIV-1 DNA load of <10 copies/million PBMCs within 13 months of birth. Rapid decay of HIV-1 DNA was observed in adults treated in Fiebig stage I and II ($t_{1/2}$ of 21 days in the first 2 weeks). Thereafter the second phase decay was much slower ($t_{1/2}$ of 198 days)[30]. In children treated around 2 months of life the total HIV-1 DNA $t_{1/2}$ in the first 24 weeks of life was 53 days, where after it was 124 days between 24 and 48 weeks[22]. Another study that defined early treatment as before 3 months of life found a $t_{1/2}$ of 107 days in the first year of life[23].

The mechanism of rapid HIV-1 DNA decline to low or undetectable levels in infants treated shortly after birth is unknown and requires further investigation. Mechanisms could include the following: 1) Low numbers of pre-treatment infected cells due to maternal ART and infant prophylaxis, 2) Possible rapid loss of cells with unintegrated virus[30]; 3) high CD4 turnover with a large proportion of short-living infected CD4 cells that decay rapidly[31]; or 4) a large proportion of surviving cells with integrated HIV-1 DNA that may have defective genomes harboring deletions, and as our assay detects HIV-1 *integrase*, these genomes may not be detectable[32].

Our study had the following limitations: First, infants diagnosed at birth and who initiated on early ART were recruited from the public health sector and often only enrolled later into our study. Second, having only a DBS sample pre-ART, we could not accurately assess decay up to the first on-treatment visit, as although HIV-1 DBS DNA levels were normalized for amplifiable cell equivalents, it contained multiple cell types and there was a limited recovery of total nucleic acid, precluding a comparison with subsequent HIV-1 DNA levels measured in PBMC. This combined with not having more frequent sampling prevented us

from determining whether the earliest HIV-1 DNA decay was log-linear or biphasic. Third, adherence in young infants is challenging, evident from the high number of viremic children and some having had delayed HIV-1 RNA suppression, limiting our ability to study HIV-1 DNA decay under optimal conditions.

Despite the difficulty of treating infants, HIV-1 DNA decayed very rapidly in most infants and reached very low levels in the first year of life. As HIV is rapidly progressive in infants, it requires early diagnosis and treatment[5]. Moreover, very early ART could result in infants with small HIV reservoirs, who may be good candidates for future immunological interventions aimed at remission or cure. Rapid HIV-1 RNA and DNA decay poses a diagnostic challenge. The combined result of maternal ART (effectively intra-uterine treatment of infected babies), extensive neonatal prophylaxis and possibly some ARV absorption through breastfeeding[33], may together contribute to suppression of viral replication and result in undetectable HIV-1 DNA and RNA levels early in life and false negative diagnostic tests. It is therefore crucial that a definitive diagnosis is established as soon as possible after birth before HIV-1 DNA or plasma HIV-1 RNA become undetectable.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure.

Infants who had assay detectable cell associated HIV-1 DNA before or at treatment initiation were included. As only dried blood samples (DBS) were available from pre-treatment, which contain cells other than peripheral blood mononuclear cells (PBMC), HIV-1 cell associated DNA results shown in this figure were all from PBMCs collected after treatment initiation. HIV-1 DNA (black circles) copies/million cells and plasma HIV-1 RNA (grey triangles) copies/mL were assessed in 11 patients. “S” denotes those suppressed and “V” those viremic. Open icons indicate HIV-1 DNA or plasma RNA below the limit of detection: plasma HIV-1 RNA was censored at < 100 copies per mL and HIV-1 DNA loads at < 3 copies/million peripheral blood mononuclear cells (PBMCs). All suppressed infants except S6 reached an HIV-1 DNA level < 10 copies/million PBMCs. S9 had HIV-1 DNA declining to undetectable at 3.5 months. The decay slope of suppressed individuals had a conditional R^2 (95% CI) of 0.77(0.53–0.91); Mean $t_{1/2}$ (95% CI): 64.8 (47.9–105.7) days. V3 had a visit at 1.5 months on ART for which no blood sample was received, thereafter at 3.8 months HIV-1 DNA was undetectable. V5 had undetectable HIV-1 DNA at the first on-ART visit but had viremia detected subsequently. HIV-1 DNA levels increased in V1 and V5.

Table

Infants included in the study

Patient ID	Gender	Child PMTCT	Child PMTCT Drugs	Age(days) at ARV Start #	Age (days) recruited *
S2	Male	Yes	Immediate cART	0	35
S3	Female	Yes	NVP, AZT	3	18
S4	Female	Yes	AZT/3TC	5#	7
S5	Male	Yes	NVP, AZT	7	22
S6	Female	Yes	Immediate cART	0	30
S7	Female	Yes	NVP, AZT	6	12
S9	Male	Yes	NVP, AZT	8	8
V1	Female	Yes	NVP	6	22
V3	Male	Yes	NVP, AZT	8	54
V4	Male	Yes	NVP, AZT	7	20
V5	Female	Yes	NVP, AZT	4	10

A 2–5 day delay between initial dual therapy and triple therapy. The initial combination antiretroviral regimen in all was azidothymidine (AZT), lamivudine (3TC) and nevirapine (NVP). NVP was replaced by lopinavir/ritonavir (LPV/r) at a corrected age of 42 weeks; AZT was replaced by abacavir (ABC) at approximately 3 months of age. “S” indicate virologic suppressed infants and “V” viremic infants.

* On the recruitment day the first PBMC samples to investigate HIV-1 reservoirs were collected.