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Evaluation of HIV-1 resistance to antiretroviral drugs among 150 patients after six months of therapeutic interruption

ORIGINAL RESEARCH ARTICLE

Evaluation of HIV-1 resistance to antiretroviral drugs among 150 patients after six months of therapeutic interruption

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Summary: Most of the antiretroviral (ARV) studies in Brazil have been reported in treatment-experienced and naive patients rather than in the setting of treatment interruption (TI). In this study, we analysed reasons given for TI and resistance mutations occurring in 150 HIV-1-infected patients who underwent TI. Of the patients analysed, 110 (73.3%) experienced TI following medical advice, while the remaining patients stopped antiretroviral therapy (ART) of their own accord. The main justifications for TI were: ARV-related toxicities (38.7%), good laboratory parameters (30%) and poor adherence (20%). DNA sequencing of the partial *pol* gene was successful in 137 (91.3%) patients, of whom 38 (27.7%) presented mutations conferring ARV resistance. A higher viral load prior to TI correlated with drug resistance ($P < 0.05$). Our results demonstrate that there are diverse rationales for TI and that detection of resistant strains during TI most likely indicates a fitter virus than the wild type. High viral loads coupled with unprotected sex in this group could increase the likelihood of transmission of drug-resistant virus. Thus, treating physicians should be alerted to this problem when the use of ARVs is interrupted.

Keywords: human immunodeficiency virus, HIV, antiretroviral therapy, resistance, treatment interruption

INTRODUCTION

Brazil has the second highest number of HIV-1 cases in the Americas, after the USA, with an estimated number of 730,000 HIV-1/AIDS cases at the beginning of 2008 (2008 Report on the Global AIDS Epidemic). In 1996, the Brazilian Ministry of Health (BMH) adopted a policy for controlling the HIV-1 epidemic by providing antiretroviral (ARV) drugs for all HIV-1-infected patients who needed therapy. Subsequently, national AIDS mortality has fallen by 50% and HIV/AIDS hospitalizations have fallen by 70–80%.[1] In addition, because of the increase in access to antiretroviral therapy (ART), from approximately 38,000 patients in 1997 to 180,000 in 2006,[2] the average survival time of Brazilian patients with HIV/AIDS seeking medical care has increased from less than six months to at least five years (The World Health Organization Report, 2004).

The successful use of ARV drugs did not come without increased cost and substantial side-effects that may jeopardize the success of current therapeutic options.[3] Besides treatment toxicities, there are several factors that contribute to treatment failure, including viral genetics, adherence to drug regimens

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and emergence of drug-resistant variants.[4,5] Evidence for dissemination of drug-resistant variants in Brazil stems from reports of primary resistance in recently infected individuals (0–12.7%), as well as those with longstanding infections (5%).[5–8]

Difficulties encountered in treated patients are well known and include problems due to emergence of drug-resistant strains and decreased tolerance to side-effects of therapy. This has led to changes in the guidelines for initiating treatment. Updates from the International AIDS Society – USA recommend initiation of ART for symptomatic patients with established disease, regardless of CD4 cell count, and for all asymptomatic individuals and those with specific conditions and co-morbidities with CD4 counts less than or equal to 500 cells/μL.[9]

Some scientists have proposed to evaluate structured treatment interruption (TI) in individuals to avoid the complications involved in the administration of continuous ART.[10–13] Although TI was never explicitly recommended by the treatment guidelines from the BMH, it became a frequent and inevitable option used by Brazilian clinicians before the results from SMART[14] and other studies were published.[15,16]

Although ART has been provided without charge to all HIV-1-infected individuals, much of the focus on ARV resistance in Brazil has been limited to treatment-naive patients, therapy-experienced patients or persons who have been infected with resistant virus rather than acquiring it following TI.[8,17,18] A previous unpublished study conducted in 2001 in São Paulo, Brazil, aimed at evaluating drug use and drug

resistance in 170 HIV-infected outpatients found that approximately 10% of these patients eventually switched to long-term TI (Jamal L, personal communication). The prevalence and reasons for TI in Brazilian HIV-1-infected patients with treatment failure and multidrug-resistant virus are largely unknown. Prompted by these needs, we designed this cross-sectional study to better understand the reasons for TI in routine clinical practice and the consequences in 150 HIV-infected adults who had stopped taking treatment for at least six months. We also attempted to explore whether the various justifications for TI, together with clinical, laboratory and epidemiological variables, are linked to the emergence of genotypic drug-resistance mutations.

MATERIALS AND METHODS

Patients

We enrolled 150 HIV-1-infected patients who were admitted to the 'Casa da AIDS', an outpatient clinic, and Sao Paulo State STD/AIDS Reference and Training Center (CRT-DST/AIDS), Brazil, between April 2002 and June 2005. The two sites are responsible for the care of four to five thousand HIV-infected patients. Eligibility criteria were restricted to adults at least 18 years of age who had been on combination ART for more than six months and had interrupted their therapy for at least six months upon study entry. The median time between the start of TI and genotype testing was 338 days, with a range of 130–532 days. After providing written, informed consent, participants were interviewed about their sociodemographic and epidemiological status. Clinical data, laboratory values and types of ART received throughout their care were acquired by reviewing the medical records of all participants. This study was approved by the Institutional Ethical Research Board in the CRT-DST/AIDS and Hospital das Clinicas da FMUSP.

Nucleic acid extraction, amplification and sequencing

Regardless of viral loads, DNA was extracted from patients' peripheral blood mononuclear cells (PBMCs) using the QIAamp blood kit (Qiagen GmbH, Hilden, Germany) as per the manufacturer's instructions. HIV-1 partial *pol* proviral DNA was amplified from purified genomic DNA by the polymerase chain reaction (PCR) as previously described.[8] Both DNA complementary strands were sequenced directly from purified PCR products by using a variety of internal sequence-specific primers, fluorescent-dye terminators and *Taq* polymerase on an automated sequencer (ABI 3100, Applied Biosystems, Inc, Foster City, CA, USA). Data from the sequenced fragments were edited, assembled into contiguous sequences with minimum overlap of 20 bp and a minimal 85–100% mismatch, and a consensus of both strands was generated by the Sequencher program (Gene Code Corp, Ann Arbor, MI, USA). The sequence was submitted to the Stanford HIV-SEQ programme (http://hivdb.stanford.edu)[19] where it was interpreted as having low-, intermediate- or high-level resistance to the individual drugs. Briefly, the programme compares user-submitted protease and reverse transcriptase (RT) sequences with a consensus reference sequence and uses the nucleotide distances as query parameters for interrogating the viral RT and protease sequence database.

Statistical analysis

Data were entered and validated with EpiData version 3.1 (EpiData Association, Odense, Denmark). Qualitative parameters were given as proportions (percentages). Differences were calculated using the chi-square test for proportions and the non-parametric test (Mann-Whitney U test) for quantitative analysis. Statistical analyses were performed using Stata version 9.0 software (StataCorp, College Station, TX, USA). Test results were considered significant for a two-sided P value <0.05.

RESULTS

The study group was composed of 150 HIV-1-infected participants, of whom 92 (61.3%) were men (median age, 37 years, range 22–63 years). Most male participants (77; 51.3%) were men who have sex with men (MSM) and only nine subjects had a history of injection drug use. The number of patients who had unprotected sexual contact during the 12 months prior to the study was 56 (37.3%). A total of 130 (86.7%) of the subjects had had sex with two or more partners. All participants but one had at least six years of formal education. The median time between the diagnosis of HIV infection and the cessation of therapy was 6.2 years (range 2.7–7.8 years). The median time on ART was 4.1 years (range 2.5–5.8 years), and 108 (72%) individuals had been treated with two or more regimens. An AIDS-related opportunistic disease was diagnosed in 55 (37.0%) patients previously, of whom 35 had a TI planned by their physician. The reasons for TI within this group were: medication side-effects in 18 patients, satisfactory laboratory response in nine patients, poor compliance to ART in five patients and more than one reason in three patients. After treatment cessation, AIDS-related illnesses developed in five out of the 55 AIDS cases and in only one out of the 95 (63%) AIDS-free participants.

Although plasma HIV-1 RNA remains the material of choice for the determination of drug-resistant mutations and guiding therapeutic decisions[20,21] the proviral PBMC DNA sequence can contain a variety of multiple archived mutations that are not present in plasma, particularly among patients undergoing TI or those with previous episodes of treatment failure due to selection of mutations found in the cellular compartments.[22,23] This, combined with the stability of DNA compared with RNA, and the fact that HIV DNA recovered from the proviral compartment can reliably be used for the determination of drug resistance mutations in patients receiving ART[24–28] influenced our decision to use proviral DNA in this study.

Of the 150 HIV-1-infected participants, samples from 137 (91.3%) were amplified and the protease/RT genes sequenced, enabling subtype classification and drug resistance. The HIV-1 subtypes of the studied subjects were distributed in the following manner: 123 clade B (89.8%), nine clade F1 (6.6%), four B/F recombinant strains (2.9%) and two clade C (1.4%). As shown in Table 1, drug resistance-associated mutations did not significantly correlate with the year of ART initiation, previous and last ART regimen or drug adherence.

Analysis of TI among the 137 subjects showed that 101 (73.7%) of the patients had interrupted treatment following medical advice while the remaining stopped of their own accord. The reasons given for TI were: 56 (40.8%) had ARV-related side-effects, 40 (29.2%) had satisfactory laboratory responses and/or started therapy based on criteria that were no longer used, 28 (20.4%) had poor adherence, 11 (8.0%) had two

Table 1 Treatment characteristics and antiretroviral resistance among the successfully genotyped samples in 137 patients during therapeutic interruption

| Characteristics | ARV resistance | | | |
	No n (%)	Yes n (%)	P value	No
Year of ART initiation			0.2	
1981–1996	26 (68.4)	12 (31.6)		38
1997–2001	73 (74.5)	25 (25.5)		98
Number of previous ART regimens			0.9	
1	27 (71.0)	11 (28.0)		38
2	25 (71.4)	10 (28.6)		35
≥3	47 (73.4)	17 (26.6)		64
First ART regimen used			0.3	
NRTI*	78 (72.9)	29 (27.1)		107
NRTI + NNRTI	5 (100.0)	0		5
NRTI + PI†	15 (62.5)	9 (37.5)		24
NRTI + NNRTI + PI	1 (1.0)	0		1
Last ART regimen used			0.6	
NRTI	33 (68.7)	15 (31.3)		48
NRTI + NNRTI	35 (47.5)	12 (25.5)		47
NRTI + PI	28 (71.8)	11 (28.2)		39
NRTI + NNRTI + PI	3 (100.0)	0		3
CD4 nadir (cells per mm^3)			0.1	
≤50	5 (50.0)	5 (50.0)		10
≥51–200	7 (87.5)	1 (12.5)		8
≥201	87 (73.1)	32 (26.9)		119
Previous ART interruption			0.1	
Yes	43 (66.5)	22 (33.5)		65
No	56 (77.8)	16 (22.2)		72
Decision for treatment interruption			0.4	
Medical recommendation	75 (74.3)	26 (25.7)		101
Patient's own decision	24 (66.7)	12 (33.3)		36
Reasons for interruption			0.5	
Poor adherence	19 (67.9)	9 (32.1)		28
Started ART based on criteria that were no longer used/satisfactory laboratory parameters	28 (70.0)	12 (30.0)		40
Unable to endure ART side-effects	40 (71.4)	16 (28.5)		56
Pregnant	2 (100.0)	0		2
Multiple reasons	10 (90.9)	1 (9.0)		11

ARV = antiretroviral; ART = antiretroviral therapy; NRTI = nucleoside reverse transcriptase inhibitor; NNRTI = non-nucleoside reverse transcriptase inhibitor; PI = protease inhibitor

*The vast majority of patients treated with NRTIs received a combination of two NRTIs (mostly AZT + 3TC)

†The vast majority of patients received a boosted PI

or more of the aforementioned reasons and two (1.4%) women became pregnant. Among the sequenced samples, 38 (27.7%) revealed mutations associated with resistance to at least one of the ARV drug classes. Twenty-nine of them had nucleoside reverse transcriptase inhibitor (NRTI)-associated mutations, 15 had non-nucleoside reverse transcriptase inhibitor (NNRTI)-associated mutations and only five had protease inhibitor-associated mutations (Table 2). Most of the patients exhibited few mutations, but some presented a complete pattern of drug resistance. A mutation at codon K103, which confers a high level of resistance to nevirapine, delavirdine and efavirenz, was detected in nine patients. A mutation at K103 was also found in association with M184V in one case. Additionally, the amino acid substitution M184V, which is associated with high-level resistance to emtricitabine and lamivudine and low-level resistance to abacavir, was detected in three patients, one of whom had been off treatment for at

least 28 months. Strong positive correlation between detectable viral load and drug resistance was evident before TI (*P* = 0.004) but not during therapy initiation or before the genotypic test, as shown in Table 3.

DISCUSSION

Scheduled TI has been suggested as an alternative therapeutic option to reduce both the burden of toxicity caused by prolonged exposure to ARV and costs of the medication. Recently, large cohorts like SMART, Staccato and TRIVACAN[14–16] have evaluated this strategy. The SMART and TRIVICAN studies were terminated prior to completion due to high morbidity events in the intermittent therapy group that were not detected in the STACCATO study. The absolute risk of disease progression was low in those trials, making the debate about the use of TI in current practice open for patients with difficulties in maintaining therapy.[29] Indeed, the BMH 2010 consensus has suggested TI in patients with high CD4 and low adherence to ART (*Recommendations for ART in adults and adolescents infected with HIV*. Brasília: Ministry of Health, 2010). Our results showed, even before the results of these studies were published, that Brazilian physicians had been forced to interrupt drug therapy because of unpleasant adverse effects and non-adherence to ART, even though this practice had not been indicated by the BMH at that time. However, for 30% of the patients, there were no other reasons besides satisfactory laboratory parameters, allowing them to defer therapy as per more recent guidelines.

It has been well established that emergence of resistance mutations in the absence of drug selection pressure can decrease both viral replicative capacity and fitness.[30,31] Furthermore, earlier studies have provided enough evidence of an apparent replacement of HIV-resistant isolates in plasma by wild-type viruses in a majority of patients who undergo TI after the development of resistant viruses.[11,32,33]

The systematic study of the disappearance of HIV drug resistance mutations in patients stopping treatment has only recently received attention. Trignetti *et al*.[34] found that mutations associated with NRTI resistance exhibited different dynamic rates of disappearance during TI. For instance, the disappearance of M184I/V mutations in HIV RNA was reported to occur rapidly after TI (median survival time <4.3 months) in a majority (91.5%; 87/95) of their patients and coincided with a surge of wild-type strains. In our current findings, we showed that M184 and K103 resistance mutations persisted in HIV-integrated DNA during TI in four and nine patients, respectively. However, as we do not know how many patients had these mutations at the initiation of ART, it is difficult to know the significance of this finding in the population described. It is noteworthy that in patient 29, virus expressing M184V persisted for at least 13 months after treatment cessation. This relative longevity of M184V was contrary to the usual rapid disappearance of this mutation in patients undergoing TI. It is possible that long-lived cellular compartments had been fuelled early in infection with the same mutated strains and had not been replaced.[35] Alternatively, this patient may have acquired viruses containing the M184V mutation during primary infection which makes it less likely to revert to wild type since the transmission of resistant strains is usually clonal.[36]

One of the aims of this study was to evaluate a resistance mutation pattern among these types of patients. We have asked clinicians from two HIV outpatient centres to request a

Table 2 Distribution of mutations associated with high- and low-level drug resistance in 38 long-term therapy interrupted patients who developed viral resistance

Sample number	Pre-cessation treatment regimens	Months after TI	Current mutations associated with resistance to			Current drugs to which virus is resistant based on genotype	Patient decision for interruption
			PIs	NRTIs	NNRTIs		
1.	AZT, 3TC, IDV, RTV	6	M36I	T215ST	V106I	AZT*	No
2.	AZT, 3TC	6	L33V, L63P	K70KR, K219KQ		D4T* AZT†	No
3.	DDI, D4T, IDV, LPV/r	6	V32IV, L33FL, M46I, I47IV, G73AT, I84V, L90M, L10F, L63P, I93L	M41L, E44D, D67N, L74V, V118I, L210W,T215Y, K219R,	K103S, V108I, G190A, F227FL	3TC*, FTC* ABC†, AZT†, D4T†, DDC†, DDI†, TDF†, DLV†, EFV†, NEV†, ATV†, DRV†, FPV†, IDV†, LPV†, NFV†, SQV†, TPV†	Yes
4.	AZT, 3TC, EFV	6	L63P	M184V	K103N	3TC†, FTC†, DLV†, EFV†, NVP†	
5.	AZT, 3TC, LPV/r	6	L10I, L63LPS, I93L		K103KN, P225HP	DLV†, EFV†, NVP†	No
6.	AZT, 3TC, LPV/r	6	M36I		K103N	DLV†, EFV†, NVP†	Yes
7.	AZT, 3TC, NFV	7	M36I	T215N		AZT*, D4T*, DDI*	Yes
8.	AZT, DDC	7	M36I, L63S,	K219KQ		AZT*	No
9.	AZT, DDC	8	L63P	M41LM, V118IV, L210W, T215D		ABC†, AZT†, D4T†, DDI†, TDF†	No
10.	AZT, 3TC	8	L63P, V177AITV	M41LM, T215ST		ABC*, DDI*, TDF* AZT†, D4T†	No
11.	3TC, D4T, NVP	8		M41LM, T215FIST		TDF* ABC†, AZT†, D4T†, DDI†	No
12.	AZT, 3TC	9	V77I	K70KR		AZT*	No
13.	AZT, DDI	9	K20R, M36I, L63P	T215Y		ABC*, DDI*, TDF* AZT†, D4T†,	No
14.	AZT, 3TC, EFV	9	M36I, L63LP		K238KT	EFV* DLV†, NVP†	No
15.	D4T, 3TC, EFV	9		T215X	Y188FHLY	AZT*, D4T*, DDI* DLV†, EFV†, NVP†	Yes
16.	D4T, DDI, EFV	9	K20M, M36I, I93L		K103N	DLV†, EFV†, NVP†	Yes
17.	AZT, 3TC, EFV	9	M36I, L63T	M41L, D67N,V118I, L210W, T215Y		3TC*, FTC* ABC†, AZT†, D4T†, DDI†, TDF†	No
18.	3TC, DDC, D4T	10	L63P	M184MV		3TC†, FTC†	Yes
19.	AZT, 3TC, EFV	10	L63P	D67G	K103N, G190A	AZT* DLV†, EFV†, NVP†	Yes
20.	AZT, DDI	10		M41L, L210W, T215D		ABC†, AZT†, D4T†, DDI†, TDF†	Yes
21.	AZT, DDI, LPV/r	10	L63P, A71T			AZT*, D4TV*	No
22.	D4T, 3TC, EFV	10	L63P, V77I		G190EG	DLV† EFV†, NVP†	Yes
23.	AZT, DDI	11	M36I, V77I	D67N, V118I, T215FL, K219Q	K103R	TDF* ABC†, AZT†, D4T†, DDI†	No
24.	3TC, D4T, SQV, RTV	11	I54V, V82A, L90M, L10I, K20R, M36I, L63P, A71V	M41L, L118I, L210W, T215H		DRV* ABC†, AZT†, D4T†, DDI†, TDF†, FPV†, IDV†, LPV†, NFV†, SQV†, TPV†	No
25.	AZT, DDI, LPV/r	12	D30N, L63S, A71V, I93L	T215Y		ABC*, DDI*, TDF* AZT†, D4T†, NFV†	Yes
26.	AZT, DDI	12	L63P	M41L, T69N, V118I, L210W, T215C		3TC*, FTC* ABC†, AZT†, D4T†, DDI†, TDF†	No
27.	AZT, 3TC, EFV	13	K20R, M36I		K103N	DLV†, EFV†, NVP†	No
28.	3TC, D4T, NFV	13	D30N, N88D, L63P, A71V, V77I			NFV†	Yes
29.	3TC, D4T	13		M184V		3TC†, FTC†	No
30.	AZT, DDI	14	L63P	D67G, K70R, L210F, K219Q		AZT†, D4T†	No
31.	AZT, 3TC, LPV/r	15	M46L, L10F, V77I, I93L		K103N, P225H	ATV*, FPV*, IDV*, LPV* NFV†, DLV†, EFV†, NVP†	Yes
32.	AZT, 3TC, EFV	16	V77I, I93L	M184G	Y188H, G190D	3TC†, EFV†, DLV†, NVP†	No
33.	AZT, DDI	16	M36I, L63LP	M41LM, T215ST		ABC*, DDI*, TDF* AZT†, D4T†	No
34.	3TC, D4T	16	L63P, A71V, I93L	K103N		DLV†, EFV†, NVP†	No
35.	AZT, 3TC	22	M36I, L63P	D67N, K219Q		D4T* AZT†	No
36.	AZT, DDI	27	V77I	M41L, T215L		ABC*, DDI*, TDF* AZT†, D4T†	No
37.	AZT, 3TC, NVP	40		M41L		AZT*, D4T*	No

NRTI = nucleoside reverse transcriptase inhibitor; NNRTI = nonnucleoside reverse transcriptase inhibitor; PI = protease inhibitor; AZT/ZDV = zidovudine; 3TC = lamivudine; EFV = efavirenz; IDV = indinavir; D4T = stavudine; DDI = didanosine; DLV = delavirdine, NVP = nevirapine, ABC = abacavir, TDF = tenofovir, FTC = emtricitabine, SQV = saquinavir, FPV = fosamprenavir, TI = treatment interruption

*Minor mutations

†Major mutations

viral genotypic resistance test in patients who were off treatment for more than six months.

Because our study was based on a convenience sample, we were neither able to define how frequent drug interruption in individuals with chronic HIV infection is occurring in these clinics nor able to analyse individuals who stopped visiting the clinics. Another limitation of this study was that we could not obtain a sample before TI. Therefore, we do not know the

Table 3   Association of viral load and CD4 count according to genotypic resistance at 3 different periods

Periods*	Viral load (copies/mL)			CD4+ count (cells/mm^3)		
	<400 *n* (%)	≥400 *n* (%)	*P* value	<200 *n* (%)	≥200 *n* (%)	*P* value
At treatment initiation						
Sensitive	2 (4.0)	48 (96.0)	0.4	5 (6.7)	70 (93.3)	0.2
Resistant	0 (0.0)	17 (100.0)		4 (17.8)	23 (85.2)	
Prior to therapy interruption						
Sensitive	47 (50.0)	47 (50.0)	0.004	3 (3.1)	93 (96.9)	0.4
Resistant	8 (22.2)	28 (77.7)		2 (5.7)	33 (94.3)	
Prior to genotypic test						
Sensitive	3 (3.2)	90 (96.8)	0.2	4 (4.3)	89 (95.7)	0.3
Resistant	0 (0.0)	37 (100.0)		3 (8.1)	34 (91.8)	

*Totals for each period may be less due to missing data

prevalence of resistance in this group under drug selection. Nevertheless, we have evaluated actual patients who have stopped their treatment on the base of medical recommendations due to ART side-effects and toxicities.

Overall, we found that 27.7% of the patients in this group harbour a drug-resistant strain. Besides viral load detectability before drug interruption, no other variables could be correlated with drug resistance. The pattern of resistance varied from few to a complete set of mutations. This is notable because the median time between TI and the genotype testing was 338 days, allowing for one of many different patterns to emerge as a major species in these individuals and suggesting these HIV variants are stable, at least in PBMCs, and have similar fitness to the wild-type virus. The viral load of these patients rose to values similar to those before treatment, making it more likely for them to transmit the virus. This fact, combined with the history of unprotected sex, renders these patients a risk for transmitting stable HIV-resistant strains.

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