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Drug-Associated Resistance Mutations in Plasma and Peripheral Blood Mononuclear Cells of Human Immunodeficiency Virus Type 1-Infected Patients for Whom Highly Active Antiretroviral Therapy Is Failing

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In 32 patients for whom highly active antiretroviral therapy was failing, a good agreement between drug resistance-associated mutations in plasma and peripheral blood mononuclear cells (PBMCs) was found (k = 0.85). The mutations with the lowest agreement were 20R, 63P, and 84V in the protease gene and 184V in the reverse transcriptase gene. In eight patients, primary drug resistance mutations were detected only in PBMCs.

The presence of resistant viral strains is routinely verified in plasma samples only, since circulating virus variants are considered representative of the viral population escaping the drug pressure. Nevertheless, archival human immunodeficiency virus (HIV) DNA present in PBMCs might represent the reservoir of additional drug-resistant viral variants (8).

This study was designed to assess the level of agreement between the drug resistance-associated mutations in plasma and peripheral blood mononuclear cells (PBMCs) in 32 patients for whom highly active antiretroviral therapy (HAART) was failing.

A commercial kit was used to identify mutations in the *pol* gene of HIV type 1 (HIV-1) (ViroSeq HIV-1 V2 genotyping system; PE Biosystems, Foster City, Calif.). DNA was extracted from 3×10^6 PBMCs with a High Pure PCR template preparation kit (Roche Diagnostics GmbH, Mannheim, Germany).

The Cohen k test was used to determine the correlation between the presence of HIV-1 drug resistance in plasma and PBMCs. Cohen k agreement is defined as poor if k is ≤ 0.20, fair if k is ≥ 0.21 and ≤ 0.60, substantial if k is ≥ 0.61 and ≤ 0.80, and good if k is >0.80 (5). The association between determinant factors and discordance in plasma and PBMC genotypic analysis was assessed by crude and adjusted odds ratios (OR) and their 95% confidence intervals (CI) through univariate and multivariate models.

Patients received a mean of 5.5 antiretroviral drugs (range, 3 to 8) during a mean of 57 months of treatment (range, 9 to 126). Twenty-eight (87%) and 16 (50%) of 32 subjects were exposed to protease inhibitors (PI) and nonnucleoside reverse transcriptase inhibitors (NNRTI), respectively.

In 492 (21.9%) of 2,240 codons analyzed, there was evidence of drug resistance. In plasma and PBMC genotypic analysis, means of 7.4 (±4.7 [standard deviation]) and 7.9 (±5.5) drug resistance mutations were detected, respectively. When total numbers of mutations were calculated for each sample, they were higher in plasma than in PBMCs in 9 of 32 samples (28%), higher in PBMCs than plasma in 14 of 32 samples (43%), and the same in plasma and PBMCs in 9 of 32 samples (28%). A significant correlation between the time of the last ART and the number of protease (PR)-related drug resistance mutations detected in plasma ($r = 0.42$; $P = 0.024$) and in PBMCs ($r = 0.52$; $P = 0.006$) was found. No significant correlation between mutations in the reverse transcriptase (RT) gene in PBMCs and plasma and the time of drug exposure was detected.

The agreement values (mean Cohen k) between drug resistance mutations in plasma and PBMCs are shown in Table 1. The mean of the k values for each codon was 0.85 (range, 0.32 to 1). Codons with the lowest k values in the PR gene were 20R, 63P, and 84V, whereas that in the RT gene was 184V. All NNRTI-related mutations (103N, 108I, 181C, 188H, and 190A) had good agreement ($k > 0.80$).

Primary drug resistance mutations were detected in PBMCs but not in plasma from 8 of 32 patients. With regard to the RT gene, the following mutations were reported: 184V in three cases, 41L in two cases, and 103N and 215Y in one case. With regard to the PR gene, 82A was detected in two cases and 84V and 46I were detected in one case. In all these patients, the discordant mutations archived in PBMCs were likely to be selected by previous ART.

Univariate analysis showed no significant difference in terms of demographic, virologic, and immunologic parameters between the 8 patients with discordant genotypic PBMC-plasma analysis and the remaining 24 patients with concordant genotypes (Table 2).

A significantly longer period of ART (OR, 0.23; 95% CI, 0.059 to 0.87) and higher number of NRTI drugs (OR, 0.29; 95% CI, 0.09 to 0.95) were reported for patients with discor-

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TABLE 1. Agreement values between primary and secondary HIV-1 drug resistance mutations detected in plasma and PBMCs

Mutations	Mean *k* value		
	PR gene	RT gene	PR and RT genes
Primary	0.77	0.87	0.84
Secondary	0.80	0.91	0.86
All	0.79	0.89	0.85

dant genotypes than for patients who showed a concordant assay. Upon multivariate analysis, no parameter was confirmed to be statistically significant.

A substantial correlation between HIV-1 drug resistance mutations detected in plasma and PBMCs of patients for whom HAART was failing was detected in this study. Nevertheless, PBMC HIV sequences showed a wider variety of mutations than viral sequences from plasma.

Plasma HIV RNA represents the aliquot of actively replicating virus selected by the antiretroviral regimen administered (4). In this respect, a significantly more homogeneous virus population in plasma during treatment may not be surprising. PBMC HIV DNA reflects archival proviral sequence with the widest genetic diversity, in which all the changes accumulated over time during the subsequent treatment schedules can be detected together with the wild-type viral strain (7).

In this study, the primary mutations related to NNRTI resistance showed a good agreement ($k > 0.80$) in the two biological compartments analyzed. Previous reports showed that subjects with primary HIV infections had a high prevalence of transmission of HIV-resistant strains (2, 9) and of NNRTI-resistant isolates (6). A possible explanation of these findings is that HIV-1 strains with the primary mutations related to NNRTI resistance (6) show no significant difference in terms of replication capacity with respect to the wild-type virus. Therefore, a sustained viral fitness could explain the transmission of drug-resistant virus and the presence of the same mutational pattern in different compartments.

In agreement with another study (1), the virus carrying the 184V mutation had one of the lowest agreement values ($k = 0.625$) between the two compartments. Several reports demonstrated an impaired fitness of isolates with 184V related to reduced RT processivity and increased fidelity (10, 11) and to reduced pyrophosphorolysis (3).

In this study, the majority of discordant primary mutations in the PR gene were detected only in PBMCs. Specifically, the four patients with PI-related drug resistance mutations de-

TABLE 2. Demographic and clinical parameters of 32 patients for whom HAART was failing, stratified according to the agreement of primary drug resistance-associated mutations in PBMCs and plasma

	Result for patients with genotypic plasma-PBMC analysis outcome		OR (95% CI)	*P*
	Discordant ($n = 8$)	Concordant ($n = 24$)		
Age[a]	42 ± 12	36 ± 8	0.92 (0.82–1.03)	0.17
No. of patients				
Male	7	18	1.89 (0.19–19.02)	0.58
IDV	2	8	1.50 (0.19–13.82)	0.51
Homosexual	3	8	0.83 (0.12–5.94)	0.57
Heterosexual	3	9	1 (0.15–7.05)	0.66
Time on ART, months[a]	80 ± 31	50 ± 29	0.23 (0.059–0.87)	0.03
No. of CD4 cells pre-ART, cells/μl[a]	179 ± 114	186 ± 172	0.78 (0.23–2.65)	0.69
HIV RNA level pre-ART, copies/ml[a]	5.96 ± 5.89	5.55 ± 5.57	0.55 (0.18–1.67)	0.29
Time on last HAART, mo[a]	18 ± 13	19 ± 9	1.42 (0.49–4.09)	0.51
CD4 at time of genotyping, cells/μl[a]	263 ± 225	276 ± 229	1.77 (0.56–5.52)	0.33
HIV-RNA at genotype, copies/ml[a]	5.16 ± 5.33	5.48 ± 5.93	1.00 (0.35–2.85)	1.00
No. of patients with:				
NRTI exposure	8	24		
NNRTI exposure	4	11	0.85 (0.13–5.46)	0.58
PI exposure	8	19		
No. of drugs				
NRTI	4.1 ± 0.9	3.2 ± 0.9	0.29 (0.09–0.95)	0.042
NNRTI	0.6 ± 0.7	0.6 ± 0.7	0.68 (0.19–2.35)	0.55
PI	1.7 ± 1.0	1.5 ± 1.0	0.68 (0.29–1.56)	0.36
All	6.4 ± 1.8	5.23 ± 1.8	0.68 (0.41–1.13)	0.14
No. of patients with subtype non-B HIV-1 isolates	3 (37%)	5 (21%)	0.33 (0.05–2.36)	0.27

[a] Values are means ± standard deviations.

tected only in PBMCs (82A in two patients, 84V and 46I in one patient each) were not undergoing PI therapy at the time of the genotypic analysis but had been previously treated with PI.

In clinical practice, the presence of drug-resistant viral strains is routinely verified in plasma samples. However, archival HIV DNA present in PBMCs might represent the reservoir of additional drug-resistant viral variants that could emerge after a failing therapy is changed based on genotypes detected in plasma. Moreover, sequencing HIV DNA from PBMCs may be useful during a simplification of ART in patients with undetectable viral loads.

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