Analysis of HIV-1 Mutation Patterns in Patients Failing Antiretroviral Therapy

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The emergence of mutations encoding drug resistance is supposed to be a significant limitation to the clinical efficacy of inhibitor compounds directed against specific HIV-1 enzymatic targets. We have used a commercial test (Visible Genetics Inc., Paris, France) to study the prevalence of mutations occurred in HIV-1 protease and reverse transcriptase (RT) genes in 93 HIV-1 infected patients treated with at least one regimen containing a protease inhibitor (PI) and failing to the current therapeutic regimen. Protease mutations conferring resistance to at least one PI were detected in 46/93 (49.4%) of strains, 25 (26.8%) of which showed resistance to all PIs. Reverse transcriptase mutations conferring resistance to at least

one RT inhibitor were detected in 57/93 (61.2%) of strains, 18 (19.3%) of which showed resistance to all RT inhibitors. The most frequent RT mutations were T215Y/F, M41L, and M184V (41.9, 40.8, and 40.8%, respectively), while L63P, L10R/V, and A71V/T (58, 41.9, and 34.4%, respectively) were the most represented protease substitutions. We have found no mutations encoding for multiple dideoxynucleoside resistance (Q151M or T69SS). Twelve of our patients (12.9%) had no mutation encoding drug resistance and were completely sensitive to all RT and protease inhibitors. Therefore, not all virological failures are caused by HIV-1 genomic resistance. *J. Clin. Lab. Anal.* 15:43–46, 2001. © 2001 Wiley-Liss, Inc.

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INTRODUCTION

The development of sensitive and reverse transcriptase PCR methods to quantify genomic viral RNA in patient plasma has proven to be of the greatest value in assessing the efficacy of HIV antiretroviral therapy (1,2). Highly active antiretroviral therapy (HAART) has demonstrated the ability to induce a marked decrease in HIV-1 viral load (3,4) and the current standard of treatment consists of combinations of three or more drugs to achieve maximal and durable viral suppression, which is the primary goal of therapy (5). Soon after the introduction of HAART, its advantages over potent antiretroviral therapies has been tempered by the results of several trials. Complete suppression of viral replication in the long term is realistic in only 50–60% of patients treated with triple combinations regimens in clinical practice (6–8).

Recently, the virologic outcome of therapy has been correlated with changes in the RNA genome of HIV-1 and the research is focused on the measurement of drug resistance with both genotype and phenotypic methods (9,10). Although the information provided by these tests is becoming increasingly useful to the selection of alternative regimens in case of failure (11,12), studies about epidemiology and frequency of mutations encoding resistance are still scarce.

Briefly, drug resistance is the evolutionary result of the reverse transcriptase process which is highly error-prone (13). Viral particles with adaptive advantages on adverse environments (drug pressure or immune response) are selected and will overgrow wild-type strains (14). Interpretation of genotypic-resistance test results requires an appreciation of the panel of those mutations that are selected by various antiretroviral drugs, as well as of the potential for cross-resistance to other drugs conferred by some of these mutations. On the other hand, HIV is highly polymorphic and it is therefore not possible to predict the viral sequence found in any infected patient.

The results of Viradapt and GART studies (15,16) indicate that the short-term virologic response to therapy is significantly greater when results of resistance testing are available if compared to clinical standard of care.

The aim of this study is to assess the prevalence of muta-

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tions occurring in the HIV-1 genome of 93 HIV-1-infected patients who were previously treated with at least one regimen containing protease inhibitors and who did not benefit from their current therapeutic regimen.

MATERIALS AND METHODS

Subjects

Subject studies were HIV-1-infected multidrug-experienced patients who did not benefit from their current therapy. In our study population ($n = 93$), 60 patients (64%) were taking AZT, 40 (43%) d4T, 13 (14%) ddI, 20 (21.5%) ddC, 67 (72%) 3TC, 6 (6.4%) NVP, 2 (2.1%) EFV, 27 (29%) SQV, 5 (5.3%) NFV, 36 (39%) IDV, and 6 (6.4%) were taking RTV. Criteria of failure include: (1) less than 0.5–0.75 log reduction in plasma HIV-1 RNA by 4 weeks of therapy; (2) failure to suppress plasma HIV-1 RNA to below the limit of detection (50 c/ml) within 4–6 months of therapy; and (3) a threefold increase of HIV-1 RNA from nadir.

Resistance Testing

When more than 2,000 RNA copies/ml were present in the blood sample (HIV-1 Quantiplex branched DNA, bDNA Chiron Bayer Diagnostic), RNA was extracted by the QIAamp Viral RNA Kit (Qiagen GmbH, Germany), then retrotranscribed and amplified according to the manufacturer's instructions of Tru Gene™ HIV-1 sequence-based assay (Visible Genetics Inc., Paris, France). Briefly, a 1.3-K sequence is generated with a single tube RT-PCR technique. After that, protease and transcriptase gene coding for the two main targets of drug treatments were bi-directionally sequenced using a sensitive single-tube chemistry, called CLIP™ Visible Genetics Inc.) which generates a sufficient quantity of fluorescently labelled terminated copies of DNA to enable sequencing. The method uses two different dye-labelled primers for each of the three sequencing reactions: protease (codons ≤ 1 to ≥ 99), RT beginning (codons ≤ 39 to ≥ 143) and RT middle (codons ≤ 138 to ≥ 244). Samples were then loaded onto the sequencer and electrophoresis was performed. Negative and positive controls were always included. The negative control consisted of plasma from HIV-1-negative blood donors and the positive controls was noninfectious transcribed RNA from a plasmid containing HIV-1_{BH10}. Finally, the generated sequences were compared to a database containing the wild type strain HIV-1_{lav-1} and known drug-resistant mutations present in the HIV-1 RNA were highlighted by software provided by Visible Genetics Inc.

RESULTS

Ninety-three patients met the inclusion criteria and were recruited for the study which lasted from March, 1998 to January, 1999. Twelve of our patients (12.9%) had no known drug

resistance encoding mutations and were therefore classified as being genotypic sensitive to all RT and protease inhibitors. But 7 of the 93 patients (7.5%) had accumulated mutations that conferred resistance to all RT and protease inhibitor compounds. Genotypic strains resistant to all protease inhibitors were identified in 25 (26.8%) of our patients, while mutations conferring resistance to all RT inhibitors were identified in 18 (19.3%) failing subjects. Finally, genotypic strains resistant to at least one protease inhibitor or RT inhibitor resulted in 47 (50.5%) and 57 (61.2%) patients, respectively (Table 1).

When the entire sequences (99 positions) of the protease genes were analysed, we found substitutions in 43 (43.4% of the protein) amino-acid positions in comparison to the wild type. Only 18 have been described as involved in the development of drug resistance. Mutations at protease position 63 were the most represented in our patients (58%) followed by mutations at position 10 (41.9%) and position 71 (34.4%).

In the RT gene, 60 mutated positions were found (18 encoding drug resistance). The most frequent mutations were T215 Y/F, M41L, and M184V (about 40% each). Out of the 6 more frequent mutations found in the RT gene, 5 encoded zidovudine resistance. In 7th and 8th frequency positions (13 and 10.7%) we found mutant codons at positions 181 and 103 which have been associated to non-nucleoside RT inhibitors resistance (Table 2).

Lamivudine resistance mutation M184V was detected in 38/93 (40.8%) patients; 34/38 (89.5%) of them were taking the drug when the resistance test was performed. Zidovudine resistance mutation T215Y/F was detected in 39/93 (41.9%) patients, 21 of whom (53.8%) were taking the drug when the resistance test was performed.

Primary protease inhibitor-resistance mutations were detected at a lower rate than secondary mutations: I50V mutation was never observed, D30N was detected in two patients taking nelfinavir, and G48V was sequenced in nine patients. Mutations that encoded resistance to saquinavir and nelfinavir are shown in Table 3.

TABLE 1. Genotypic sensitivity/resistance to HIV-1 therapy according to genotype interpretation^a

	RT gene (n = 93)	Protease gene (n = 93)	RT + Protease (n = 93)
Genotypic sensitivity to all drugs in the class	18 (19.3%)	21 (22.5%)	12 (12.9%)
Genotypic resistance to at least 1 drug in the class	57 (61.2%)	47 (50.5%)	74 (79.6%)
Genotypic resistance to all drugs in the class	18 (19.3%)	25 (26.8%)	7 (7.5%)
Total	93 (100%)	93 (100%)	93 (100%)

^aRT inhibitors, d4T, 3TC, ddI, ddC, AZT, DLV, NVP, and EFV; P inhibitors, IDV, RTV, SQV, and NFV.

TABLE 2. Mutations frequency into protease and reverse transcriptase gene

Region gene	Position	Frequency	Ratio (%)
Protease	63P	54/93	58
	10R/V	39/93	41.9
	71 V/T	32/93	34.4
	90 M*	31/93	33.3
	82A/F/T*	29/93	31.1
	77I	24/93	25.8
	46I/L*	22/93	23.6
RT	54V/L	20/93	21.5
	215Y/F*	39/93	41.9
	41L*	38/93	40.8
	184V*	38/93	40.8
	210W	27/93	29
	67N	25/93	26.8
	70R*	19/93	20.4
	219Q	15/93	16.1
	181C	12/93	12.9
	103N/R	10/93	10.7

*Primary mutations associated to resistance.

DISCUSSION

The emergence of mutations encoding drug resistance is a significant limitation to the clinical effectiveness of drugs targeted against specific HIV-1 enzymes (11,12). The rapid generation of HIV-1 variants in an infected person constitutes a natural mechanism for biological adaptability in adverse environments (14,17).

The particular genotypic sequence and pattern of mutations in previously treated patients is believed to be somehow drug specific and often patient specific. Because of the relatively limited number of antiretroviral agents and the limited availability of resistance testing in clinical practice (19,20), epidemiological studies of mutations distribution (18,21) can be used by HIV-1/AIDS specialists to complement information provided by CD4 cell count and viral load. It is important to note that the most frequent mutations we have found in the RT gene are considered to be primarily associated to resistance (T215Y, 41.9%; M41L, 40.8%; M184V, 40.8%). On the other hand, the most frequent mutations found in the protease gene (L63P, 58%; L10R/V, 41.9%; A71V/T, 34.4%) are secondarily associated to resistance, as already pointed out by Yahi et al. (18).

TABLE 3. Primary and secondary mutations distribution correlated to saquinavir and nelfinavir resistance

	Saquinavir resistance 46/93 (49.4%) patients	Nelfinavir resistance 38/93 (40.8%) patients ^a
Primary mutations	36/46 (78.3%) ^b	2/38 (5.3%) ^c
Secondary mutations	10/46 (21.7%)	36/38 (94.7%)

^aSixteen additional patients had 1 or 2 secondary mutations that, combined to a further substitution, confer resistance to nelfinavir.

^bPresence of 90V and/or 48V codons.

^cPresence of 30N codon.

High-level resistance to protease inhibitors usually develops after a large number of mutations is sequentially accumulated and, also, it takes a relatively long time to emerge “in vivo.” In contrast, resistance against RT inhibitors often develops rapidly because only a single substitution is required. Thus, we have found no mutation encoding for multiple dideoxynucleoside resistance (Q151M or T69SS) (22,23). Despite the difference in the “genetic barrier” between RT inhibitors and PIs, we found that 18 (19.3%) of 93 patients were resistant to all available RT inhibitors and that the frequency of resistance to all available protease inhibitors increases to 26.8% (25/93), which is probably due to the cross-resistance between protease inhibitors.

Mutations encoding for zidovudine resistance are the most frequently detected (18,23), reflecting the widespread use, since the late 1980s, of this drug in suboptimal combinations for the treatment of HIV-1 infection. Prevalence of RT codon 215 mutation was as high as 42% in 1996 in Spain, a value very similar to ours from this study, although it was detected in antiretroviral naive patients (24). The rate of persistence of T215Y/F and M184V mutations, concomitant or not with current AZT or 3TC regimens, respectively, demonstrates that M184V mutation does not persist after lamivudine is discontinued while T215Y/F mutation remains, even after treatment is changed. One important result of our study is the low frequency of mutations associated with resistance to non-nucleoside analogue RT inhibitors (NNRTI).

Thirty-eight out of 93 patients (40.8%) tested resistant to nelfinavir, but in only 2 (5.3%) was the mutation D30N (primary mutation) present—both were taking nelfinavir when resistance was tested. The remaining 36 patients (94.7%) had a secondary mutations pattern which confers resistance to nelfinavir, in spite of the fact that none of the patients had never taken the drug. Comparison of primary versus secondary saquinavir resistance mutations is quite different: 36 (78.3%) of the 46 patients resistant to saquinavir showed a primary mutation and the remaining patients had secondary mutations. Our data support the utility of resistance assays in identifying patients that will benefit from salvage therapy.

Nowadays, HIV-1 drug-resistance assays are achieving widespread use in clinical practice making antiretroviral decision-making simpler and more effective for the patient (12,25). Nevertheless, not all virological failures are caused by viral drug resistance (12.97% of our patients had no genotypic drug-resistant viruses). Genotypic/phenotypic viral resistance to antiretroviral drugs is an important, but not the only, reason for treatment failure. Other factors such as drug pharmacokinetic/pharmacometabolism or patient compliance have to be considered in order to explain HAART failure. Individual variation on drug absorption, distribution, metabolism, excretion, protein-binding effect, and drug-to-drug interactions may influence clinical drug effectiveness (12,26). Reduced adherence to therapy caused by increased complexity, pill burden, and toxicity is probably the single greatest contributor to treatment failure (27,28).

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