HIV-1 Genotype Resistance Pattern and Evolution in Patients Failing Nelfinavir-Containing Regimens

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Clinical and in vitro studies have suggested that nelfinavir (NFV)-containing regimens may not preclude the use of other protease inhibitors (PIs) in treatment sequencing. We have studied the prevalence of 30N mutation in a human immunodeficiency virus-1 (HIV-1)-infected cohort and the virological response to a PI-containing regimen in patients who had previously failed NFV. A total of 335 patients were included in the study; 32 of them were antiretroviral-naïve and 303 were antiretroviral-experienced (251 were PI-experienced). Mutations 30N and/or 90M were not detected in sequences obtained either from the antiretroviral naïve or non-PI-experienced patients. The 30N mutation was detected in 21/251 (8.3%) of PI-

experienced patients and 90M in 103/251 (41%). Moreover, we have observed that the 88D and 77I mutations were present in more than 75% of patients harbouring the 30N HIV-1 variant and the 71T mutation was present in almost 50% of them. Finally, mutations 30N+90M were never detected together in the same HIV-1 strain. The 30N and 90M mutations were not observed together. The presence of mutations at positions 36, 46, 71, 77, and/or 88 in a 30N background, increases the risk of the cross-resistance to other PIs. The use of NFV as a first-line PI, as an application of drug sequencing strategies, may help preserve future PI options. *J. Clin. Lab. Anal.* 19:26–29, 2005. © 2005 Wiley-Liss, Inc.

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INTRODUCTION

Potent antiretroviral therapeutic combination has changed the natural history of human immunodeficiency virus-1 (HIV-1) infection from an almost invariably fatal outcome to a chronic disease, but as antiretroviral agents are unable to eradicate infection (1,2), prolonged clinical benefit requires the continuous use of an active antiretroviral regimen. However, the majority of HIV-1-infected individuals receiving care in clinical practice are likely to experience virologic rebound within 3 years of initiating therapy (3).

Drug resistance is associated with the selection of viral mutants that are able to replicate under the pressure of a specific antiviral agent. Preventing the emergence of drug-resistant strains of HIV-1 is thus essential to avoiding virologic rebound and preserving future treatment options. The process of applying knowledge of resistance patterns to the selection of initial and subsequent therapy is known as “sequencing antiretroviral drugs” (4).

The use of protease inhibitors (PIs) is usually associated with mutations (primary mutations) conferring

cross-resistance to all of this class of drugs; the continuous use of PIs after virological failing prunes the virus for accumulation of new mutations (secondary mutations) with ongoing replication and increase cross-resistance (5,6).

In vitro studies have suggested that nelfinavir (NFV)-containing regimens may not preclude the use of other PIs in the treatment sequencing, however this observation was mainly based on the sole consideration of primary mutations at position 30 and 90 of the HIV-1 protease gene (7,8). Moreover, it has been suggested that 90M and 30N mutations in the protease gene, both associated with NFV-containing regimens failure, are mutually exclusive (7,8).

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Although PI resistance mutations were not identified in a majority of patients failing their first PI-based triple regimen containing NFV in a study Nunez et al. (9), and other reasons for failure must be ruled out, usually 30N mutation, rather than 90M mutation is recognized in almost all cases of genotypic NFV resistance (8,9). In addition, the frequency of 30N acquisition is higher in patients who receive NFV as an initial PI than in those who receive NFV as an alternative (10).

We retrospectively analyzed our database to evaluate the prevalence of 30N HIV-1-resistant mutants and its association with other mutations that cause broad PI cross-resistance. Moreover, we studied the virological response to a PI-containing regimen in patients who had previously failed NFV.

MATERIALS AND METHODS

Data were collected from HIV-1-positive patients in the outpatients clinic of the Institute of Infectious and Tropical Diseases of Brescia (Italy) between January 2000 and June 2001. Resistant testing was performed after virological failure of a regimen including antiretroviral therapy (plasma HIV-1-RNA >1,000 copies/mL; Versant™ HIV-1 RNA version 3.0 assay (Bayer Corp., Tarrytown, NY) or in few patients before initiating their first antiretroviral therapy.

For genotypic analysis of resistance-associated mutations, TRUGene HIV-1 sequence-based assay (Visible Genetics, Paris, France) was used. Briefly, a 1.3-kb sequence from the pol region, encompassing the entire protease gene and the major part of the reverse transcriptase gene of HIV-1, was reverse transcribed and polymerase chain reaction amplified in a single tube. Bidirectional sequencing ladders were generated from complementary DNA amplicons by CLIP sequencing technology and were detected in a MicroCell (Visible Genetics), using the MicroGene Clipper (Visible Genetics) automated sequencer. Chromatograms for the sequencing reactions were analysed using GeneObjects software (Visible Genetics), assembled and compared with the wild-type HIV-1_{HXB2} sequence using GeneLibrarian (Visible Genetics). The accuracy of all sequences was verified by manual proofreading.

RESULTS

Patient Characteristics

A total of 335 patients, 250 (75%) males and 85 (25%) females from whom a HIV-1 resistance test was available were included in the study. A total of 32 (9.5%) patients were antiretroviral-naïve and 303 (90.5%) were taking antiretroviral drugs at the moment of the resistance test; from this last group, 251 (82.8%)

were PI-experienced patients (199 patients [59.4%] were failing a first-line antiretroviral regimen) and 52 (17.1%) had not previously used any PIs. At the time of testing, 34/303 patients (11.2%) were receiving a first-line of antiretroviral therapy; 168/303 (55.4%) were receiving a second- or third-line therapy and 101/303 (33.3%) were heavily pretreated patients (more than four antiretroviral regimens).

Among PI-experienced patients, the most frequent amino acid substitutions in the protease (PRO) gene (>20%) were observed at positions: 10 (48.8%), 36 (29.7%), 46 (27.7%), 54 (39.6%), 71 (36.9%), 77 (25%), 82 (47.6%), and 90 (40.8%).

Prevalence of 30N Mutation: Relationship With Other Mutations and Drug-Selective Pressure

A total of 87 patients included in this study were NFV-experienced (35 patients were using NFV as their first PI at the moment of performing the resistance test), but the 30N mutation was present only in 21/87 (24.1%) of them; 18/21 of them (85.7%) were failing a regimen including NFV. Treatment failure occurred within 8–26 (mean 13) months after treatment with NFV was initiated. Three patients who carried the HIV-1 mutant with the 30N mutation had used NFV in the previous regimen (mean time from stopping NFV: 11 months). Mutation at position 30N was not detected in the HIV-1-protease sequence (PRO) obtained either from the antiretroviral naïve or non-NFV-experienced patients. Therefore, the 30N mutation was only observed in NFV-experienced patients.

The 90M mutation was present in 103/251 patients (41%) that had used PIs. The presence of 90M was observed in both experienced and inexperienced patients for each PI (Table 1). Moreover, 30N+90M mutations were not present at the same time in any of our patients.

Finally, we analysed the PRO mutational pattern in patients harbouring 30N-HIV-1 variants, (Table 2) and we observed that 88D and 77I substitutions were present in more than 75% of patients harbouring 30N HIV-1 variants and that 71T was present in almost 50% of them.

Virologic Response to a PI-Containing Regimen in Patients Who Had Previously Failed NFV

A total of 28 NFV-experienced patients initiated a new PI-containing regimen after failing NFV. Virological response was observed in 19 of them (67.8%) (undetectable viral loads <500 copies/mL after 6 months of therapy). The most frequent mutations in the 10 patients who were nonresponders were 90M (80%) and 46I (70%). Two patients (2/19; 10.5%) did

TABLE 1. Prevalence of 30N or 90M mutations according to PI drugs experience

	Prevalence of 30N (n = 21)		Prevalence of 90N (n = 103)	
	Experienced	Nonexperienced	Experienced	Nonexperienced
Saquinavir	12 (57.1%)	9 (41.8%)	89 (86.4)	14 (13.5%)
Ritonavir	5 (23.8%)	16 (76.1%)	13 (12.6)	90 (87.3%)
Indinavir	13(61.9%)	8 (38%)	70 (67.9)	33 (32%)
Nelfinavir	21 (100%)	0 (0%)	9 (8.7%)	94 (91.2%)

TABLE 2. Frequency of 30N-associated mutations on PRO sequences (according to the International AIDS Society (13))

Mutation	Frequency
36I	4 (19%)
37N	21 (100%)
46L	4 (19%)
63P	21 (100%)
71T	10 (47.6%)
77I	16 (76%)
88D	16 (76%)

not have mutations in the PRO gene. Baseline mutations prior to switching in responders patients were as follows: 63P (19/19; 100%), 30N (12/19; 63%), and 88D (10/19; 52.6%).

DISCUSSION

Proteases encoded by retroviruses are highly specific enzymes that are critical for the stage of viral replication and, therefore, they have been an attractive target for drug development. However, the HIV-1 protease gene has been shown to be more mutation-prone than expected and consequently, the development of viral resistance to PIs has become a major problem in clinical practice (11,12).

Sequencing strategies that take into account the varied mutational patterns that may emerge after exposure to particular antiretroviral agents may help to maximize the number of effective antiretroviral combinations available and thus improve the long-term durability and potency of antiretroviral therapy. The effectiveness of a second PI in patients who failed an initial regimen containing drugs from this class is unclear but believed to be low (8,9). Some studies have shown that patients who failed a NFV-containing regimen can respond to a new regimen with a new PI (8,9).

The 30N and 90M mutations in the PRO gene are considered primary mutations conferring the loss of susceptibility to NFV (13). While 30N occurs solely in patients receiving NFV and confers no in vitro or

clinical cross-resistance to all PIs currently approved for use, the 90M mutation either contributes to or directly confers resistance to all PIs currently approved for use (13). Therefore, the emergence of one or the other mutation may have a different impact on the design of subsequent therapeutic regimens (14).

In our study, we observed that the 30N mutation was present only in NFV-experienced patients, with a prevalence of 24%, while the 90M mutation was more frequent (almost 50% of PI-experienced patients) and that the 90M mutation was extensively distributed among patients who had used all PI drugs. Our data confirm that the 30N mutation, rather than 90M mutation, is recognized in almost all cases of genotypic NFV resistance (8,9). In addition, the frequency of 30N acquisition is higher in patients who receive NFV as an initial PI than in those who receive NFV as an alternative (10).

Finally, in the present study we did not find any HIV-1 strain carrying the 30N+90M mutations together; this is also a quite rare association in the clinical setting. Several combinations of mutations in the PRO gene have been reported to demonstrate low protease activity and reduced viral fitness, but 30N+90M is the most incompatible mutation pair described (7). The exact mechanism of the incompatibility between 30N and 90M is not understood. The double mutation causes almost complete elimination of infectivity and replication capacity and can be regarded as being a virtually mutually exclusive combination (7).

Here, we present that 30N mutations are predominant after NFV failure; 90M mutations are frequent after failure of any PI, both mutations are rare in the same strain, and we show that almost 70% of patients can respond to a new PI after NFV failure.

As previously demonstrated (3), since the development of resistance to antiretrovirals is almost the rule over the long-term, the issue of sequential use of the different options in an appropriate manner is the great relevance. The use of NFV as a first-line PIs, as an application of drug sequencing strategies, may help preserve suppressive options over time that include other PIs.

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