Long-Term Benefit of Genotypic-Guided Therapy and Prevalence of Multinucleoside Resistance in an Italian Group of Antiretroviral Multiexperienced Patients

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Multiple nucleoside resistance involves specific genetic changes in the HIV-1 reverse transcriptase gene, such as Q151M mutation and an insertion of two serine aminoacids at RT codon 69. Among 432 patients failing antiretroviral therapy, five (1.15%) harboured viruses with Q151M mutation into the RT gene and no viruses were identified harbouring insertion at codon 69. Also we have studied the long-term benefit of HIV genotypic testing with the failure to reach a viral load below 50 copies/ml within 1 year of antiretroviral therapy using as the primary end-point. A group of 64 HIV-positive antiretroviral multiexperienced patients were examined, all of them failing the current ART.

HIV-RNA changed -0.8 log at month 4 and $+0.1$ log and -0.5 log at months 8 and 12, respectively. The proportion of patients with viral load below 50 copies/ml was 19.3, 32.8, and 28.1% at months 4, 8, and 12, respectively. In multidrug-experienced patients, genotype-guided therapy is not in fact able to achieve complete viral suppression in more than 30% of patients after 1 year of ART. The development of more precise resistance tests and interpretations are needed for better control of HIV replication. Other metabolic/pharmacokinetics factors of poor drug adherence should also be assessed. *J. Clin. Lab. Anal.* 15:127–130, 2001. © 2001 Wiley-Liss, Inc.

Key words: genotyping; HIV multiexperienced

INTRODUCTION

Complete and prolonged suppression of HIV replication is a primary objective of antiretroviral therapy. Patients who achieve undetectable viral loads can be expected to have a more sustained antiviral effect (1,2). The availability of a new generation of ultrasensitive viral load assays has demonstrated that patients whose viral load has reached values lower than 20–50 copies/ml have more durable responses than those whose nadir is below 200 copies/ml (3,4). Major factors contributing to loss of suppression include suboptimal drug potency, inadequate drug exposure, and insufficient regimen adherence (5,6).

For patients with incomplete viral replication suppression, the emergence of resistance to components of the current drug regimen and cross-resistance that limit the effectiveness of future therapies are the main problems. Therefore, resistance tests can improve our ability to select subsequent treatment regimens, and actually, genotypic and phenotypic resistance assays are considered important tools with which to choose more effective salvage regimens (7). However, failure of genotypic-/phenotypic-driven treatments after a variable span of time is, unfortunately, quite common in multidrug-experienced HIV-infected patients (8,9).

On the other hand, the main clinic problems of the resistance test are the correct interpretation of the HIV mutations and the difficulty in making regimen decisions when the presence of multidrug resistance mutations limits subsequent therapeutic options. In particular, few mutations into reverse transcriptase sequences can confer resistance to all antiretroviral nucleoside analogues acting as reverse transcriptase inhibitors (10). The emergence of this multiresistant virus is particularly worrying when current guidelines recommend two nucleosides RT inhibitors combined with another drug from a different class of antiretroviral drugs (protease inhibitors or non-nucleoside RT inhibitors) as the standard protocol for HIV treatment.

It has been shown that ZDV/ddI or ZDV/ddC combination therapy can select a multidrug-resistance (MDR)-mutation, the substitution Q151M. It results in high-level resistance of ZDV, ddI, ddC, d4T, and partial cross-resistance to 3TC. Also,

Grant sponsor: Istituto Superiore di Sanita; Grant number: 30B.14.

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Received 13 October 2000; Accepted 5 December 2000

a set of four new additional mutations (A62V, V75I, F77L, and F116Y) may be acquired into RT gene following a defined sequence to improve Q151M-HIV fitness (11,12).

More recently, an insertion at RT codon 69 of 6 bp (T69S-SS) has been described as a marker mutation of multinucleoside resistance (13).

Several studies have been designed to examine short-term efficacy of genotypic-guided therapy but few data are available about long-term benefits of HIV-1 resistance testing in multidrug-experienced patients. Only in the VIRADAPT study are results presented after 12 months of follow-up after using genotypic-resistance assays to manage HIV-infected patients, using the failure to reach HIV-RNA value below 200 copies/ml as primary endpoint (14).

Here, we have studied the long-term benefit of genotypic testing, using as the primary endpoint the failure to reach a viral load below 50 copies/ml within 1 year of therapy, and using the prevalence of the substitution Q151M or insertion T69S-SS as markers of genotypic multidrug resistance to nucleoside analogues in our HIV positive cohort.

MATERIALS AND METHODS

Our total HIV-positive cohort includes 1,342 subjects, 946 of which are drug experienced with at least two nucleoside analogues. Their current therapeutic regimen includes (or does not) drugs from other classes (protease inhibitors or non-nucleoside analogues acting as reverse transcriptase inhibitors). In 432 multinucleoside experienced patients who failed to benefit from the current therapeutic regimen, sequencing-based HIV-1 genotyping was done to determine the presence of multinucleoside resistance. Also, in a group of 64 antiretroviral multiexperienced patients, there was a median of 3.4 nucleoside analogues (range 2–5) for > 12 months and a median of 1.4 protease inhibitors (range 1–4) for > 6 months. The virologic and immunologic benefit of a genotype-guided treatment was evaluated every 4 months during one year of follow-up.

To this aim, plasma was collected and RNA extracted by QIAamp Viral RNA Kit (Qiagen GmbH, Germany) then retrotranscribed and amplified according to the manufactured instructions of Tru Gene HIV-1 sequence-based assay (Visible Genetics Inc. [VGI], Paris, France).

Briefly, a 1.3-K sequence is generated with a single-tube RT-PCR technique. After that, protease and transcriptase genes coding for the two main targets of anti-HIV drug were bidirectionally sequenced using a sensitive single-tube chemistry, called CLIP (VGI) which generates 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).

Then, samples were loaded onto the sequencer and elec-

trophoresis was performed. Negative and positive controls were always included. The negative control consisted of plasma from HIV-negative blood donors and the positive control was a noninfectious transcribed RNA from a plasmid containing HIV-1_{BH10}.

Finally, the sequences generated were compared to a database containing the wild type strain HIV-1_{1av-1} and known drug resistant mutations present in the HIV-1 RNA were highlighted by software provided by VGI. Interpretation of the mutations was established according to the consensus statement on antiretroviral drug-resistance testing (15).

Viral-load determination was performed by a commercial test with a cut-off of 50 copies/ml (Quantiplex branched DNA, bDNA Chiron, Bayer Diagnostic).

RESULTS

The prevalence of Q151M mutation into HIV-1 RT gene in our group was 1.15% (5/432). In 3/5 patients (60%), Q151M mutation was associated with at least two of the additional mutations which are acquired sequentially to improve virus fitness (Table 1). In the other two patients, Q151M was not found to be associated to those typically related mutations.

Four of the five patients had been previously treated with the ZDV/ddI combination and in the fifth patient, this nucleoside combination was included into his current regimen.

We could not demonstrate any cases with the two serine insertion at codon 69 of RT gene (T69S-SS), although a large polymorphism at that position has been observed (T69S/T/I/D).

In the 64 antiretroviral multiexperienced patients observed over 12 months, genotypic-guided therapy showed a clear virologic (decrease in HIV RNA level) and immunologic (increase in CD4 cells) benefit. Baseline mean plasma HIV-1 RNA was 153,787 copies/ml and mean CD4 cell count was 241/mm³. Mean change in plasma HIV-1 RNA and CD4 cell count from baseline to month 12 are shown in Table 2. HIV-1 RNA decrease was more marked at month 4 (mean difference, -0.8 log) than at months 8 and 12 (mean differences, $+0.1$ log and -0.5 log respectively). Twenty-three of 64 patients responded to the treatment with at least 1 HIV RNA measurement below the ultrasensitive assay replication limit of 50 copies/ml.

TABLE 1. Therapeutic and virologic characters of patients harboring Q151M mutation

Patient	Number of NRTI experienced	AZT/ddI experienced (months)	Additional mutations Q151M associated
1	3	Yes (8)	F77L, F116Y
2	4	Yes (12)	None
3	4	Yes (1)	None
4	3	Yes (4)	A62V, F77L, F116Y
5	4	Yes (9)	F77L

TABLE 2. HIV-1 RNA and CD4 cell count evolution after genotypic-guided therapy

	Baseline	4 months	8 months	12 months
Viremia (log)	153.787c/ml (5.2)	25.896c/ml (4.4)	33.857c/ml (4.5)	10.412c/ml (4)
CD4 cells/ul	241	275	291	324

The proportions of patients with viral load below 200 copies/ml were 29% (18/62, but in 2 patients this value was not available), 34.3% (22/64), and 32.8% (21/64) at months 4, 8, and 12, respectively. When the 50 copies/ml limit is considered, the proportions are slightly reduced to 19.3% (12/62), 32.8% (21/64), and 28.1% (18/64) at the same time intervals. (Fig 1).

Repeated detection of virus in plasma after initial suppression to undetectable levels was observed in two patients at month 8, and in four patients at month 12 (6/64 patients 9.3%). No association with baseline HIV-1 RNA values or with a number of previous antiretroviral drugs taken has been observed.

Finally, a mean increase in CD4 T lymphocytes during the study was progressively observed from 241 cells/ul at baseline, 225 cells/ul at month 4, 291 cells/ul at month 8, and 324 cells/ul at month 12.

DISCUSSION

Recently, a complex mutation pattern was identified that is associated with high levels of resistance to all nucleoside reverse transcriptase inhibitors. The main mutation is Q151M, but accumulation of mutations at other codons is required for this broad resistance (62V, 75I, 77L, and 116Y) and they appear to improve HIV replication capacity.

The prevalence of the MDR-mutations until now has been reported to be low but quite variable, ranging from 2–19% in patients previously treated with multiple nucleoside analogues (16–18). In Europe the prevalence is reported to be lower than in America. Maeda et al. (11) describe a frequency of MRD mutation of 17–19% in their American cohort although in another American cohort studied by Lader et al., no evidence of Q151M has been reported (15).

Fig. 1. Proportion of patients with viral load below 200 copies/ml and below 50 copies/ml at baseline and months 4, 8, and 12.

Recently, in a multicenter study that involved centers in nine European countries, Q151M was identified in 1.6% of the samples and SS-insertion at codon 69 was identified in 0.5% of the study samples (19). It remains to be determined if the prevalence of the MDR mutation is dependent upon the duration and numbers of analogue nucleoside exposure.

Furthermore, the transmission of MDR-strains remains low (evidence based on studies of prevalence for RT genotypic markers of antiretroviral resistance in newly infected cohorts) (20). Our data show that in our HIV-positive cohort, Q151M mutation is also quite rare and T69S-SS insertion has not been detected. Transmission of drug-resistant viral variants to newly infected individuals, and therefore to drug-naïve persons, is a matter of serious public health consequence (20). If the prevalence remains at this level, it is expected that the transmission of such variants, and therefore their presence in newly HIV-1-infected patients, is scarce.

Finally, because the development of MDR strains substantially limits subsequent therapeutic options, the analysis of drug-resistant mutations using tests that do not permit detection of MDR-mutations is not advised. When a multinucleoside resistance is suspected, the mutation Q151M and insert at position 69 must be examined by sequencing analysis (18,21).

Although many studies carried out in treatment-experienced patients have shown a strong association between the presence of genotypic or phenotypic drug resistance and failure of the current antiretroviral treatment regimen to suppress HIV replication, few studies have evaluated the long-term virologic response to genotypic-guided therapy. Usually, these studies used the threshold of 200 copies/ml as marker of virologic failure (8,14).

The VIRADAPT study has shown that 29, 32, and 30.4% of patients receiving genotypic-guided therapy reached HIV-1 RNA levels below 200 copies/ml at months 3, 6, and 12, respectively. These values are very similar to those from our results. Such information is useful when reaching values lower than 200 copies/ml of HIV-RNA is the objective of the therapy. If HIV-RNA below 50 copies/ml is considered "complete" viral suppression, then the number of patients that reached that goal did not decrease further except at month 4 (19.3 vs. 29%) although it is maintained stable at months 8 and 12 (32.8 vs. 34.3% and 28.1 vs. 32.8%, respectively). This could be due to the fact that HIV needs more than 4 months of efficient therapy to suppress replication below 50 copies/ml as previously described (2). In our study this result is independent of baseline HIV-RNA level.

In our study, as previously demonstrated in multidrug experienced patients, we found that genotype-guided therapy is not able to achieve complete viral suppression in more than 30% of patients after 1 year of therapy regardless of the detection threshold used (50 or 200 copies/ml) (8,14).

Our results demonstrate that the proportion of virologic response to genotypic-guided therapy between different studies is quite similar when mutation interpretation is based on

the same rules (22) and when sequencing techniques are similar among different cohorts; therefore, other common factors are responsible of therapeutic failure.

During the last year, efforts have been made to describe new mutations and interactions among them, to improve the interpretation of sequencing results, and to standardize technical aspects of sequencing assays. Future studies that apply these new strategies are needed to newly evaluate the consequences for genotypic-guided therapy.

In any case, other factors must be taken into account in the effort to reach complete viral suppression in this large group of patients because current resistance tests are imperfect predictors of drug responses, and because resistance mutations may accumulate for as long as the virus replicates under drug pressure. Cellular mechanisms of resistance, pharmacokinetics, factors resulting in suboptimal drug concentrations, and poor drug adherence should also be considered.

Also, the development of more precise monitoring tools that lead to the detection of minor variants, more information about the replication capacity of the mutated viruses and, mainly, more knowledge of mutations (single and associated) that confer resistance to correctly interpret the genotype assays are needed for better prediction of virological responses to drug regimens.

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