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An additive/substractive genotypic score as a determinant of the virological response to didanosine in HIV-1 infected patients

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Abstract

Objective: To assess the genotypic determinants of the virological response (VR) to didanosine (ddI) in nucleoside reverse transcriptase inhibitors (NRTI)-experienced patients.
Methods: Human immunodeficiency virus type 1 (HIV-1) genotype was determined at baseline in 74 ddI-naïve-patients with baseline viral load >500 copies/ml and receiving ddI as part of a new regimen. VR was defined as a plasma HIV-1 RNA <50 copies/ml after three months on ddI. NRTI resistance mutations associated with higher or lower frequencies of VR with a p-value < 0.25 were retained in different sets of mutations, where the mutations associated with a worse VR were added, whereas the mutations associated with a better VR were substracted. The most significant mutation scores were then studied in a multivariate analysis.
Results: Changes at three codons (M41L, L210W, T215Y/F/D/C/E) were associated with a worse VR and three mutations (K70R, M184V, K219Q) with a better VR. The strongest association with the VR was obtained with the score M41L + L210W + T215Y/F/D/C/E − K70R − K219Q. The score was independently associated with the VR in the multivariate analysis.
Conclusion: Taking into account the mutations associated with a better VR may improve genotypic resistance algorithms. Our results are of interest for the management of antiretroviral therapy in NRTI-experienced patients.

Keywords: HIV drug resistance; Didanosine; Algorithm; Nucleoside RT Inhibitors

1. Introduction

The clinical and biological benefits of antiretroviral therapy can be limited by the selection of human immunodeficiency virus type 1 (HIV-1) drug-resistant variants (Hirsch et al., 2003). Resistance to nucleoside reverse transcriptase inhibitors (NRTI) has been shown to be mediated through the emergence of mutations in the targeted reverse transcriptase (RT) enzyme. Didanosine (ddI) resistance was firstly shown to be specifically related to the L74V mutation (St Clair et al., 1991), promoting resistance through a decrease of the incorporation of ddATP, the active metabolite of ddI. The mutation K65R can also code for resistance to ddI and to most NRTI with the exception of zidovudine, with an increasing prevalence in the last years (Parikh et al., 2004; Zhang et al., 1994). Other pathways contributing to the emergence of multinucleoside resistant viruses are also involved in resistance to ddI. The Q151M mutation, usually co-selected with changes at codons 62, 75, 777 and 116, is related to resistance to zidovudine (AZT), stavudine (d4T), ddI, abacavir and zalcitabine

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(Iversen et al., 1996; Shirasaka et al., 1995). Amino acids (most frequently dipeptides) insertions between RT codons 69 and 70 have been shown to be implicated into resistance to all NRTI (Larder et al., 1999; Masquelier et al., 2001; Winters et al., 1998). These two latter pathways are however rare, about 2% for each, in NRTI-experienced patients (Masquelier et al., 2001; Van Vaerenbergh et al., 2000). Another group of mutations is more frequently involved in resistance to NRTI; these thymidine analogue mutations (TAMs: M41L, D67N, K70R, L210W, T215Y/F, K219Q/E) are frequently selected by AZT and d4T (Izopet et al., 1999; Larder and Kemp, 1989; Pellegrin et al., 1999) and some of them have also been shown to be involved into resistance to abacavir (Brun-Vezinet et al., 2003) and tenofovir (Masquelier et al., 2004; Miller et al., 2004). The involvement of the TAMs in resistance to ddI has been postulated by a study with a limited number of patients (Costagliola, 2001). Given the high frequency of TAMs in NRTI-experienced patients with virological failure, we thought important to describe their role, as well as the importance of the other NRTI resistance mutations, for resistance to ddI. Genotypic interpretation algorithms are of importance to better define the indications of ddI in NRTI-experienced patients, since the biological cut-off values for reduced ddI susceptibility have been shown to be very close to the detection limit of most phenotypic assays. Pursuing this objective we studied the genotypic determinants of the virological response to ddI-containing regimens in a cohort of NRTI-experienced patients.

2. Patients and methods

2.1. Study population

The patients included in this retrospective study were HIV-1 infected patients followed-up at the Bordeaux University Hospital within the Aquitaine Cohort of the Groupe d'Epidémiologie Clinique du SIDA en Aquitaine (GECSA). Patients were included if they had experienced a virological failure on antiretroviral therapy, defined as plasma HIV-1 RNA >500 copies/ml, during 2000 and 2001, were NRTI-experienced but ddI-naïve, and had received ddI during at least three months as part of the subsequent regimen. No cut off for the maximum value of baseline plasma HIV-1 RNA was considered as an inclusion criteria. Patients on treatment interruption at the time of virological failure were excluded from the study.

2.2. Genotypic resistance analysis

The HIV-1 RT and protease genotypic resistance analysis was performed on baseline ddI plasma samples, obtained less than two months before starting the ddI-containing regimen and without change of the antiretroviral therapy between the date of sampling and the start of ddI. Sequence analysis was performed according to the ANRS consensus protocol using a Beckman 2000 XL sequencer, as previously described (Merel et al., 2001). The details of the methods appear at www.hivfrenchresistance.org. The genotypic analysis could be successfully performed on samples with plasma HIV-1 RNA >500 copies/ml. RT and protease resistance mutations were reported as listed by the International AIDS Society-USA panel (www.iasusa.org, update October 2004), including revertant mutations at RT codon 215.

2.3. Plasma HIV-1 RNA quantitation

HIV-1 RNA levels in patient plasma samples were determined using bDNA Quantiplex assay version 3.0 (Chiron Bayer, Emeryville, CA), with a limit of detection of 50 copies/ml.

2.4. Statistical analysis

The definition of the virological response (VR) was a plasma HIV-1 RNA <50 copies/ml at month 3 (M3) on the ddI-containing regimen. Conversely, the definition of virological failure (VF) was a plasma HIV-1 RNA $\geq$ 50 copies/ml at M3. We analyzed the impact of the presence of each mutation associated with resistance to NRTI on response, by comparing the proportion of VR in patients with and without the mutation. For comparison of qualitative variables, we used Pearson Chi square or Fisher test and Student's test or non-parametric Kruskall–Wallis test for quantitative variables. Mutations for which the p-value was lower than 0.25 in the above univariate analysis were retained for further analysis. In order to construct mutation scores, we scored at (+1) the mutations associated with VF and at (−1) the mutations associated with VR. We thus calculated the sum of positive and negative scores of each mutation retained in the univariate analysis for each patient, and evaluated different combinations of mutations in the univariate analysis.

To assess whether or not the genotypic scores were independent predictors of response, we performed a stepwise logistic regression accounting for baseline variables found to be associated with VR with p-value < 0.25 among treatment history, treatment co-prescribed with ddI, baseline viral load, CD4 count, presence or absence of non-nucleoside-RT inhibitors (NNRTI) mutations and the number of major and minor protease inhibitors (PI) resistance mutations.

3. Results

3.1. Patients baseline characteristics

Seventy-four patients with available baseline genotype and follow-up were included in the study. The baseline and treatment characteristics appear in Table 1. Included patients had a moderate pattern of treatment failure, with a median viral load at 3.9 $\log_{10}$ copies/ml [interquartile range (IQR): 3.5–4.3; range 2.3–6.1] and a relatively low number