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Genotypic resistance analyses in nucleoside-pretreated patients failing an indinavir containing regimen: results from a randomized comparative trial: (Novavir ANRS 073)

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Abstract

Background: Different studies have shown that most patients failing a first-line treatment containing a protease-inhibitor (PI) had low PI plasma levels and no PI-related resistance mutations. NOVAVIR was a randomized trial comparing stavudine/lamivudine/indinavir (d4T/3TC/IDV) and zidovudine/lamivudine/indinavir (AZT/3TC/IDV) in patients pretreated with AZT, didanosine (ddI) and/or zalcitabine (ddC) but naive for PIs.

Objective: To study the mechanisms of virological failure in NOVAVIR trial through analyses of genotypic resistance profiles of reverse transcriptase (RT) and protease (PR), and plasma IDV concentrations at time to failure.

Methods: Plasma HIV-RNA PR and RT sequences were determined in 27 failing patients (d4T/3TC/IDV $n = 11$; AZT/3TC/IDV $n = 16$) at baseline and at time to failure. IDV plasma measurements were performed in both samples.

Results: At baseline, 20 out of the 27 patients had at least two thymidine analogs associated mutations. At time to failure, mutation M184V in the RT gene was present in 22 out of the 27 failing patients. Thirteen out of the 27 (48%) patients had acquisition of PI mutations compared to baseline sequence. Of the 26 patients with adherence data, 13 (50%) subjects were classified as having difficulty in adherence. The proportion of patients with low adherence was higher in the subgroup of patients failing without acquisition of new PI mutations.

Conclusions: In patients experienced with NRTIs, failure to PI-containing regimen may occur in spite of appropriate adherence to therapy and is associated with emergence of PI mutations in half of the cases. These results suggest that, although PIs have a high genetic barrier, sub-optimal activity of associated drugs may favor the selection of PI resistance mutations.

Keywords: Mutations; Nucleosides RT inhibitors; Protease inhibitors; Resistance; Randomized trial

1. Introduction

Complete and prolonged suppression of human immunodeficiency virus (HIV) replication is a primary objective of antiretroviral therapy (Hirsch et al., 2003). Rapid and substantial reductions in viral replication should delay the emergence of resistant viruses and increase the durability of re-

Abbreviations: PI, protease inhibitor; AZT, zidovudine; d4T, stavudine; 3TC, lamivudine; ddI, didanosine; ddC, zalcitabine; IDV, indinavir; NFV, nelfinavir; APV, amprenavir; RT, reverse transcriptase; PR, protease; HIV, human immunodeficiency virus; M8, nelfinavir hydroxybutylamide metabolite; NRTIs, nucleoside reverse transcriptase inhibitors

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sponse (Kuritzkes, 1999; Hirsch et al., 2003). Major factors contributing to loss of suppression include sub optimal drug potency, inadequate drug exposure and insufficient regimen adherence.

Resistance to protease inhibitors (PI) monotherapy is characterized by sequential acquisition of mutations conferring stepwise reductions in drug susceptibility (Condra et al., 1995; Molla et al., 1996). Cross-resistance is common among PI, making the sequential use of these agents difficult (Race et al., 1999). For IDV M46I/L and V82A/F/T/S are the mutations usually described at failure but other mutations have been described leading to broad PI cross-resistance (www.iasusa.org) (Condra et al., 1996; Johnson et al., 2003).

Early reports showed that patients failing PI monotherapy or having PI added to their failing regimen had virus with multiple PI resistance mutations (Condra et al., 1995; Molla et al., 1996). Other studies have pointed out that early virological failure on a first-line triple combination therapy including PI occurs in the absence of PI-resistant mutations and with undetectable and/or low plasma antiretroviral drug levels (Descamps et al., 2000; Havlir et al., 2000; Mouroux et al., 2000).

Novavir (ANRS 073) was a prospective randomized multi center trial comparing AZT/3TC/IDV and d4T/3TC/IDV combinations in 170 patients (Joly et al., 2002) previously exposed to AZT, ddI and/or ddC but naïve for PI. The objective of our study was to determine the mechanisms of virological failure in patients enrolled in Novavir trial through analyses of PI genotypic resistance profile and plasma drug measurements at time to failure.

2. Methods

2.1. Study population

Patients randomized in the Novavir trial and who experienced a virologic failure defined by two consecutive HIV-1 RNA > 5000 copies/mL were studied. Patients had been previously treated for a median of 20 months with AZT, ddI or ddC but were naïve for other antiretroviral drugs, in particular PI. Eligible patients were randomly assigned to receive: d4T or AZT in combination with 3TC and IDV (800 mg three times daily). We studied patients experiencing virological failure within 18 months after randomization. At time to virologic failure all patients received indinavir except three patients who were switched to nelfinavir (NFV) because of IDV toxicity.

2.2. Genotypic resistance studies

Viral genotyping was performed retrospectively at baseline and at time to failure on plasma samples using the ANRS Resistance study group sequencing consensus technique as already described (Pasquier et al., 2001).

2.3. Determinations of plasma drug concentrations

Plasma samples were collected at the two points defining virologic failure. IDV and NFV plasma concentrations were measured blindly in a central laboratory using high-performance liquid chromatography coupled with an UV detection as previously described (Lamotte et al., 1999; Woolf et al., 1995). The limits of quantification were 5, 30 and 30 ng/mL for IDV, NFV and its active hydroxybutylamide metabolite (M8), respectively. Low IDV and NFV + M8 plasma concentrations were defined as concentration below or equal to 70 and 1000 ng/mL, respectively. Patients with at least one low IDV or NFV + M8 plasma concentrations were classified as having inadequate PI plasma levels and as having difficulty in adherence.

3. Results

A total of 170 patients were enrolled from March 1997 to March 1998; 85 subjects were assigned to AZT/3TC/IDV and 85 subjects to d4T/3TC/IDV. Virologic failure occurred before 18 months in 15 (18%) patients receiving AZT/3TC/IDV and in 14 (16%) patients receiving d4T/3TC/IDV. Of the 29 patients experiencing a virologic failure genotyping sequence data were available in 27 subjects and adherence data in 26 subjects.

Table 1 depicts the evolution of PI and nucleoside reverse transcriptase inhibitors (NRTIs) resistance associated mutations in 27 patients with virologic failure at baseline and at time to failure. At baseline, 20 out of the 27 patients had at least two thymidine analogs associated mutations and two had T215Y/F only. All patients had wild-type virus at codon 184 in the RT gene. At time to failure, mutation M184V in the RT gene was present in 22 out of the 27 failing patients. Thirteen out of the 27 (48%) patients had acquisition of PI mutations compared to baseline sequence. Most frequent new PI mutations occurred at codons 46 ($n = 6$), 82 ($n = 5$) and 71 ($n = 4$). Of the 26 patients with adherence data, 13 (50%) subjects were classified as having difficulty in adherence (Table 2). The proportion of patients with low adherence was higher in the subgroup of patients failing without acquisition of new PI mutations. In fact, as shown on Table 2, 11 out of the 14 patients failing with an unchanged virus compared to baseline had low PI concentrations compared to only two out of 12 patients (17%) (patients 7 and 13 on Table 1) failing with acquisition of new PI resistance mutations ($p = 0.005$).

4. Discussion

In our study, virologic failure was related to acquisition of new PI mutations in 48% of subjects. Most of these patients were adherent to treatment. The profile of mutations selected by IDV was similar to what has been reported in other studies (Condra et al., 1996; Gallego et al., 2001; Yerly