Polymorphism of the Human Immunodeficiency Virus Type 1 (HIV-1) Protease Gene and Response of HIV-1-Infected Patients to a Protease Inhibitor

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In order to analyze the impact of protease gene polymorphism on response to regimens containing a protease inhibitor, the entire protease coding domain from 58 human immunodeficiency virus type 1 (HIV-1)-infected patients who were protease inhibitor naive was sequenced before therapy was started. Plasma HIV-1 RNA levels were measured at baseline and at month 3 and month 6 after treatment. All patients were treated with a combination of two reverse transcriptase inhibitors and a protease inhibitor (saquinavir EOF [n = 28], ritonavir [n = 16], or indinavir [n = 14]). Before treatment, 30 different positions whose codons differed from the subtype B consensus sequence were observed. Major mutations associated with protease inhibitor resistance were not observed. No statistical correlation between the number of amino acid differences and the treatment efficacy at month 3 (−2.4 log) or month 6 (−2.7 log) was observed. At baseline, genotypic analysis of the HIV-1 protease gene of patients who have never received a protease inhibitor does not allow prediction of the efficacy of regimens containing a protease inhibitor.

MATERIALS AND METHODS

At baseline, the sequence of the protease gene from 58 patients who were protease inhibitor naive was characterized. Among these patients, 12 had been treated previously with a combination of two nucleoside reverse transcriptase inhibitors. Plasma HIV-1 RNA levels were measured by Roche-Monitor assay (Roche Diagnostic Systems, Inc., Branchburg, N.J.), including the ultrasensitive assay, before introduction of the protease inhibitor and at 3 and 6 months afterward (lower limit of quantification, 20 copies/ml).

All patients were treated with two reverse transcriptase inhibitors and a protease inhibitor with usual daily dosages: 28 patients received saquinavir EOF (1,200 mg three times a day), 16 received ritonavir (600 mg twice a day), and 14 received indinavir (800 mg three times a day).

HIV-1 RNA was extracted from 200 μl of plasma by using the HCV specimen preparation kit (Roche Diagnostic Systems, Inc.), reverse transcribed to cDNA, and then directly amplified by nested PCR. The primers used for cDNA synthesis and PCR amplification and cycle conditions have been previously described (4). The entire protease coding domain from the 58 patients before introduction of the protease inhibitor was sequenced on an automated DNA sequencer (Applied Biosystems model 377) and compared to the HIV-1 clade B consensus sequence (15).

Statistical analyses were performed with nonparametric tests (Spearman's rank correlation and Mann-Whitney U test).

RESULTS

Only one patient had a protease gene identical to the HIV-1 clade B consensus sequence. The protease sequences of the other 57 patients (98%) carried 1 to 9 amino acid differences from the consensus sequence. The median number of substitutions was 4; 34 patients harbored between 1 and 4 amino acid changes, and 23 had between 5 and 8 changes. A total of 30 positions differed from the consensus sequence, while the most frequent changes (prevalence, >20%) were located at positions 15, 35, 37, 41, 63, 77, and 93 (Table 1). The substitution at position 63 was particularly frequent, as it was observed in 58% of the cases. The 63P substitution (44%) represented the most common amino acid change in the protease.

The major amino acid mutations associated with reduced sensitivity to protease inhibitors, at positions 30, 48, 50, 82, 84, and 90, were not observed.

The median plasma HIV-1 RNA levels were 47,479 copies/ml before introduction of the protease inhibitor, 162 cop-
ies/ml (−2.4 log₁₀) at month 3, and 89 copies/ml (−2.7 log₁₀) at month 6 after treatment. At months 3 and 6, 7 (12%) and 26 (45%) of 58 patients, respectively, achieved complete suppression of HIV-1 RNA in plasma (<20 copies/ml).

With Spearman’s rank correlation test, no statistical correlations between the number of protease amino acid substitutions and the decrease in HIV-1 RNA levels at months 3 and 6 were observed. Moreover, the presence of the 63P mutation did not influence the evolution of HIV-1 RNA levels.

The same results were observed when the tests were performed for each drug separately (saquinavir, ritonavir, and indinavir). Moreover, the numbers of amino acid substitutions present at baseline and the response to treatment including a protease inhibitor was observed. Moreover, the presence of the 63P mutation did not influence the rate of complete HIV-1 RNA viral load suppression (HIV-1 RNA level, <20 copies/ml).

At baseline, genotypic analysis of the HIV-1 protease gene from patients who have never received a protease inhibitor does not allow prediction of the efficacy of regimens containing a protease inhibitor.

REFERENCES


