Structural modifications induced by specific HIV-1 protease-compensatory mutations have an impact on the virological response to a first-line lopinavir/ritonavir-containing regimen

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Objectives: This study evaluates the impact of specific HIV-1 protease-compensatory mutations (wild-type amino acids in non-B subtypes) on virological response to a first-line lopinavir/ritonavir-containing regimen in an HIV-1 subtype B-infected population.

Patients and methods: The prevalence of protease-compensatory mutations from 1997 to 2011 was calculated in 3063 drug-naive HIV-1 B-infected patients. The role of these mutations on virological outcome is estimated in a subgroup of 201 patients starting their first lopinavir/ritonavir-containing regimen by covariation and docking analyses.

Results: The number of HIV-1 B-infected patients with at least one protease-compensatory mutation increased over time (from 86.4% prior to 2001 to 92.6% after 2009, P = 0.02). Analysing 201 patients starting first-line lopinavir/ritonavir, the median time to virological failure was shorter in patients with at least one protease-compensatory mutation than in patients with no protease-compensatory mutations. By covariation and docking analyses, specific mutations were found to affect lopinavir affinity for HIV-1 protease and to impact virological failure. Specifically, the L10V+I13V+L63P+I93L cluster, related to fast virological failure, correlated with a decreased drug affinity for the enzyme in comparison with wild-type (ΔGmut = −30.0 kcal/mol versus ΔGwt = −42.3 kcal/mol).

Conclusions: Our study shows an increased prevalence of specific protease-compensatory mutations in an HIV-1 B-infected population and confirms that their copresence can affect the virological outcome in patients starting a lopinavir/ritonavir-containing regimen.

Keywords: antiviral, drug resistance, molecular modelling

Introduction

The HIV-1 M group shows extraordinary variation among inter- and intrasubtypes.1–4 Such diversity could influence protein function and affect the susceptibility profile of protein targets of antiretrovirals.

Among all drug classes, protease (PR) inhibitors (PIs) are the most affected by HIV-1 variability: the high number of PR amino acids substitutions strongly affects PI resistance, by reducing the inhibitors’ binding affinity for the PR enzyme (major mutations) and by restoring the replication capacity of resistant virus (compensatory mutations).5,6

The compensatory mutations L10I/V, I13V, G16E, K20I/R, M36I, D60E, I62V, L63A/P, H69K/Q, V82I, L89I/M and I93L are often wild-type (WT) amino acids in non-B subtypes and can be present at different prevalences in HIV-1 B-infected patients naive to antiretrovirals (range: 0.20%–54.0%).7 However, the observed intensification of intrasubtype HIV-1 variability8 could
increase the prevalence of these PR-compensatory mutations, to date defined as ‘genetic features of non-B strains’, also in HIV-1 B subtype.

Thus, this study aimed to evaluate: (i) the prevalence of these PR mutations over time (years) in drug-naive subtype B-infected patients; (ii) their impact on virological failure to lopinavir/ritonavir; and (iii) their impact on lopinavir binding affinity for the enzyme.

Patients and methods

Patient characteristics

This study included 3063 HIV-1 B-infected patients naïve to antiretroviral drugs, followed in several clinical centres of central Italy, undergoing genotyping resistance test from January 1997 to May 2011. Patients carrying transmitted drug resistance mutations (by population sequencing) were excluded.

Mutation prevalence

The prevalence of the PR-compensatory mutations (WT amino acids in non-B subtypes) L10I/V, I33V, G16E, K20I/R, M36I, D60E, I62V, L63A/P, H69K/Q, V82I, L99I/M and I93L was estimated in 3063 HIV-1 B subtype strains. Statistical significance (P value < 0.05) was evaluated by a χ² test for trend and Fisher’s exact test.

Fitness landscape model

In order to evaluate the potential increase in the fitness value of HIV-1 viruses containing at least one compensatory mutation in the presence of lopinavir/ritonavir over time, a fitness landscape of HIV-1 PR was estimated.

Role of PR-compensatory mutations in response to highly active antiretroviral therapy (HAART)

The correlation between the time to virological failure (defined as the first of two consecutive determinations of HIV-1 RNA level >50 copies/mL after the achievement of viral load undetectability) and the number of PR-compensatory mutations at baseline was evaluated in a subgroup of 201 patients starting their first lopinavir/ritonavir-containing HAART by using the Kruskal–Wallis test and Spearman’s rank correlation coefficient.

Mutation covariation and cluster analysis

In the set of 201 patients starting their first lopinavir/ritonavir-containing HAART, we defined positive and statistically significant correlations among PR-compensatory mutations associated or not with PI failure.

Docking experiments

For each compensatory mutation significantly associated with treatment outcome, specific mutant models were built starting from an HIV-1 [lopinavir-PR] cocrystallographic complex. To support the results obtained, we also submitted to docking simulations mutations known to cause a high level of resistance to lopinavir (I47A, L76V and V82A) and to decrease PR susceptibility to this drug (V32I and I47V).

Detailed information on the fitness landscape model and the covariation and docking analyses is available as Supplementary data at JAC Online.

Results

Frequency of PR-compensatory mutations over time in subtype B

The proportion of patients with at least one PR-compensatory mutation showed a progressive increase over time, from 86.4% in 1997–2000 to 92.6% in 2009–11 (P = 0.02) (Table 1). The linear regression model, constructed as a function of the sampling date, showed a significant increase in viral fitness from 1.37 in samples collected before 2001 to 1.49 in samples collected in the years 2009–11 (P < 0.001) (Table 1), highlighting the rising HIV-1 diversity in the last years.

This increased diversity was mainly due to the enhanced frequency of specific PR-compensatory mutations, such as L10I, G16E, I62V and I93L (Table 1).

Role of PR-compensatory mutations in response to HAART

Among the 3063 patients enrolled in this study, 201 started a first-line lopinavir/ritonavir-containing HAART and 32 of them (15.9%) failed it (patient characteristics are reported in Table S1, available as Supplementary data at JAC Online).

Evaluating the time to virological failure in the 32 patients failing their first-line HAART, we found that the presence of at least one PR-compensatory mutation at baseline correlates with a shorter time to virological failure (37.7 (27.5–74.9) weeks in patients with a least one PR-compensatory mutation versus 129 (114–143) weeks in patients without these mutations, P = 0.01). We also noticed an inverse correlation between the number of PR-compensatory mutations and the time to virological failure (Spearman’s rank test r = −0.604, P = 0.01). In particular, all of the eight patients infected with HIV-1 B with at least four PR-compensatory mutations failed their subsequent HAART in a median time of 28.0 (24.6–30.0) weeks, a time shorter than that observed in patients infected by viruses without compensatory mutations [129 (114–143) weeks, P = 0.01].

Associations among PR-compensatory mutations

To identify the potential patterns of PR-compensatory mutations affecting virological response to a first-line lopinavir/ritonavir-containing regimen, we defined the strength of association (φ) both in the set of patients failing (32 baseline samples) and in those not failing (169 baseline samples) their first HAART.

The baseline genotypes of the patients failing HAART were characterized by a significant association of L10V with either I93L (φ = 0.44) or I13V (φ = 0.27) (Figure S2A, available as Supplementary data at JAC Online). In particular, the copresence of I93L and I13V was observed in three out of four patients with L10V (bootstrap value = 0.72). These patients (all failing therapy within the 48 weeks) were infected with an HIV-1 virus also containing L63P (Figure S2B, available as Supplementary data at JAC Online).

Conversely, by analysing the baseline genotypes of the 169 patients not failing their lopinavir/ritonavir-containing regimen, G16E (prevalently found in baseline samples of patients not failing HAART; Table S2, available as Supplementary data at JAC Online) was specifically associated with L10I (φ = 0.25) and I62V (φ = 0.18) (Figure S2A, available as Supplementary data at JAC Online). The copresence of these three mutations was observed.
Table 1. Frequency of HIV-1 PR-compensatory mutations over the years

<table>
<thead>
<tr>
<th>PR-compensatory mutations</th>
<th>Frequency, n (%)a</th>
<th>P valueb</th>
<th>P valuec</th>
</tr>
</thead>
<tbody>
<tr>
<td>At least 1</td>
<td>444 (86.4)</td>
<td>0.02</td>
<td>0.01</td>
</tr>
<tr>
<td>Patients with 1 or 2</td>
<td>293 (57.0)</td>
<td>0.01</td>
<td>0.03</td>
</tr>
<tr>
<td>Patients with &gt;2</td>
<td>151 (29.4)</td>
<td>0.002</td>
<td>0.002</td>
</tr>
<tr>
<td>Number (median, IQR)</td>
<td>2 (1–3)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>L10I</td>
<td>35 (6.8)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>L10V</td>
<td>19 (3.7)</td>
<td>0.005</td>
<td>NS</td>
</tr>
<tr>
<td>I13V</td>
<td>112 (21.8)</td>
<td>0.002</td>
<td>0.02</td>
</tr>
<tr>
<td>G16E</td>
<td>27 (5.3)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>K20I</td>
<td>1 (0.2)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>K20R</td>
<td>6 (1.2)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>M36I</td>
<td>100 (19.5)</td>
<td>0.07</td>
<td>NS</td>
</tr>
<tr>
<td>D60E</td>
<td>47 (9.1)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>I62V</td>
<td>136 (26.5)</td>
<td>0.0001</td>
<td>0.0004</td>
</tr>
<tr>
<td>L63A</td>
<td>19 (3.7)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>L63P</td>
<td>295 (57.4)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>H69K</td>
<td>67 (13.0)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>H69Q</td>
<td>8 (1.6)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>V82I</td>
<td>3 (0.6)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>L89I</td>
<td>0 (0.0)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>L89M</td>
<td>10 (1.9)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>I93L</td>
<td>91 (17.7)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Landscape fitness</td>
<td>1.37 (1.21–1.57)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Only P values <0.10 are reported in the table and only P values <0.05 are reported in bold. NS, not significant.

aFrequency of PR-compensatory mutations L10I/V, I13V, G16E, K20I/R, M36I, D60E, I62V, L63A/P, H69K/Q, V82I, L89I/M and I93L was calculated for a total of 3063 HIV-1 subtype B-infected patients naive to antiretroviral drugs. Patients were collected from January 1997 to May 2011.

bP value was calculated by a χ² test for trend.

cStatistically significant differences between samples collected before 2001 and after 2008 were calculated by Fisher’s exact test for dichotomous variables and by a linear regression test for continuous variables. The Benjamini–Hochberg method was used to identify results that were statistically significant in the presence of multiple-hypothesis testing. A false discovery rate of 0.05 was used to determine statistical significance.

Figure 1. Evaluation of the AutoDock interaction energy calculated for the studied PR complexes in the presence of the PI lopinavir. The graph reports the variation of the free-energy differences (ΔG) in kcal/mol between the wild-type (ΔGwt) and the mutated (ΔGmut) HIV-1 B subtype PR complexed with lopinavir.
in four patients, with a bootstrap value of 0.86 (Figure S2C, available as Supplementary data at JAC Online).

Molecular modelling
After docking simulations and thermodynamic evaluation of each [lopinavir-PR] complex, we found that mutations preferentially correlated with failure were associated with a reduced lopinavir binding affinity if compared with the WT sequence. In particular, the drug affinity for the enzyme strongly decreased in the presence of L10V+I13V+L63P+I93L (ΔGmut = -30.0 kcal/mol versus ΔGwt = -42.3 kcal/mol), also when compared with that observed in the presence of the major lopinavir mutation I47A (Figure 1). As reported in Table S3 and Figure S3(A and B) (available as Supplementary data at JAC Online), a reduced number of contacts between the enzyme and the drug were observed in the presence of the above-mentioned mutations, if compared with WT (324, 10V+I13V+L63P+I93L versus 421wt). Specifically, lopinavir was found to lose several non-bonded interactions with PR residues L33, M36, K55, V56 and L90.

Conversely, the mutations correlated with a successful response to lopinavir/ritonavir were found to be associated with an increased lopinavir binding affinity for HIV-1 PR. In particular, in the presence of L10I+G16E+I62V, lopinavir revealed the most stabilized configuration (ΔGmut = -67.9 kcal/mol versus ΔGwt = -42.3 kcal/mol) (Figure 1). Such thermodynamic data were highlighted by the increased number of contacts observed between the drug and the mutated enzyme, if compared with WT (501L10I+G16E+I62V versus 421wt) (Table S3 and Figure S3A and C, available as Supplementary data at JAC Online). In particular, the drug established two new hydrogen bonds with the residues D25 and G48, crucial in substrate recognition.11

Discussion
In this study, we found that specific HIV-1 PR-compensatory mutations are associated with viral evolution and a faster failure of PI-based therapy in patients infected with HIV-1 subtype B.

By analysing a wide dataset of PR sequences from HIV-1 subtype B-infected patients naïve to antiretroviral drugs, the prevalence of strains with more than one PR-compensatory mutation showed a progressive increase over the period 1997–2011. The higher variability observed in the last years at positions related to PR-resistance and C, available as Supplementary data at JAC Online. In particular, the drug established two new hydrogen bonds with the residues D25 and G48, crucial in substrate recognition.11

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In particular, we observed an increased prevalence of specific PR mutations, such as L10I, G16E, I62V and I93L. To date, these PR mutations have been widely analysed, but frequently individually12–16 and their role remains controversial.14 For example, I93L was found to be strongly associated with in vivo and in vitro resistance to indinavir,15,16 I62V has been associated with virological failure in PI-treated patients17 and L63P appeared with a high frequency in patients treated with lopinavir.18 Finally, mutations at PR position 10, located in close proximity to the highly conserved residues L23 and D25, affect the conformation of these positions, thus resulting in lower PI affinity for the enzyme.11,15,20

To better characterize the role of these compensatory mutations in the field of drug resistance, we focused our attention on a subgroup of patients starting their first HAART, with a PI-containing regimen.

The first finding was that virological failure occurred more rapidly in patients with at least one PR-compensatory mutation when compared with those without such a mutation (37.7 versus 129 weeks, P=0.01). The second finding was that this difference was more evident when we stratified HIV-1 strains for the number of PR-compensatory mutations at baseline. This finding supports the concept that two or more mutations, which individually have no significant effect on a trait under selection, can, in combination, be highly advantageous or deleterious, triggering heavy modifications of the viral fitness and thus influencing the rate of disease progression.9 In particular, the combination G16E, I62V and L10I was related to a significant increase of lopinavir affinity; differently, L10V together with I13V, L63P and I93L impaired lopinavir binding affinity for the enzyme similarly to or even more than the major lopinavir resistance mutation I47A. Therefore, the combined effect of natural polymorphisms can be even more deleterious than primary mutations. This further supports the use of resistance testing, properly interpreted, in the selection of the drug components to be used in a first therapeutic regimen.

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Transparency declarations
None to declare.

Supplementary data
Detailed information on the fitness landscape model and the covariation and docking analyses, Tables S1 to S3 and Figures S1 to S3 are available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).

References