Impact of gag mutations on selection of darunavir resistance mutations in HIV-1 protease

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Objectives: To search for genetic factors in the protease and gag regions (NC-p1/TFP-p6/p6pol) involved in selection of darunavir resistance mutations.

Patients and methods: We analysed 48 protease inhibitor (PI)-experienced HIV-infected patients experiencing darunavir treatment failure. Viral genotyping at baseline and months 3 and 6 was used to assess the selection of mutations in the protease and gag regions conferring resistance to PIs.

Results: There were no genotypic differences in the studied gag region between baseline and the latest available rebound isolates. There was an association between the presence of the mutation A431V in the gag sequence and the selection of the L76V mutation in the protease sequence in the latest available rebound. The I437T/V mutation in gag and the L76V mutation in the protease were associated with a lower risk of selecting darunavir resistance mutations.

Conclusions: In these PI-treated patients experiencing treatment failure of a darunavir-containing regimen, we showed that mutations in the gag region NC-p1/TFP-p6/p6pol may influence the selection of darunavir resistance mutations; in particular, the I437T/V gag mutation that confers resistance to PIs reduces the selection of such mutations. Virus with L76V in protease or I437T/V in gag may be already resistant to darunavir and, therefore, no additional resistance mutations need to be selected.

Keywords: HIV, drug resistance, protease inhibitors, risk factors

Introduction

Darunavir is a new protease inhibitor (PI) marketed as part of a ritonavir-boosted regimen for antiretroviral-experienced patients with viral resistance mutations. Clinical trials in treatment-experienced (POWER) and in naive (ARTEMIS) patients have shown that darunavir/ritonavir has significantly better efficacy outcomes than investigator-selected control PIs. Little information is available concerning the factors associated with darunavir-selected mutations. We recently confirmed the set of emerging mutations associated with darunavir failure in PI-treated patients. Higher baseline HIV-RNA levels, smaller numbers of associated nucleoside reverse transcriptase inhibitors (NRTIs) and the wild-type codon at position 76 were associated with a higher risk of selection of darunavir resistance mutations. However, resistance to PIs seems to also involve mutations in the gag cleavage sites (CS) that may allow an increase in the enzymatic activity of the mutated viral protease and a partial recovery of the replicative capacity of viruses containing mutations in the protease.

Recent work shows that in vitro some PIs can select mutations in HIV gag comprising NC-p1/TFP-p6/p6pol (Figure 1), without selecting mutations in the protease. Nijhuis et al. confirmed the selection of mutations in this gag region in vivo in association or not with known resistance substitutions in the protease. However, it is difficult to select protease mutations in patients receiving darunavir treatment. It would therefore be interesting to look for mutations in this gag region. The study of Callebaut et al.
similarly found that this gag region is implicated in resistance to a PI with a close chemical structure to darunavir (with a bistetrahydrofurane moiety). We studied isolates from 48 PI-experienced patients with treatment failure while receiving a darunavir-containing regimen to determine whether there is any genetic determinant in the gag region (comprising NC-p1/TFP-p6/p6pol) affecting the risk of occurrence of darunavir mutations.

**Patients and methods**

**Patients and antiretroviral regimens**

We retrospectively selected 48 PI-experienced patients who had experienced treatment failure on a darunavir-containing regimen, defined as incomplete suppression of plasma HIV-1-RNA or a rebound from maximal suppression (viral load >400 copies/mL). All were treated with NRTIs and ritonavir (100 mg twice daily) plus darunavir (600 mg twice daily). Patients, of whom 89.5% were male, were infected with subtype B HIV-1 in 89.5% of cases. The median plasma HIV-1-RNA was 4.89 log_{10} copies/mL (interquartile range 4.5–5.4) at baseline, 4.25 (interquartile range 3.6–4.7) between months 1 and 3 of treatment and 4.43 (interquartile range 3.7–4.8) between months 3 and 6 of treatment. The median CD4⁺ count was 94.5 cell/mm³ (interquartile range 16–211) at baseline, 165 (interquartile range 46–281) between months 1 and 3 of treatment and 129 (interquartile range 67–259) between months 3 and 6 of treatment. The median number of PIs previously used was 4. The median numbers of antiretroviral drugs, NRTIs and non-NRTIs used concurrently with darunavir were 4, 3 and 0, respectively.

**Genotypic resistance testing**

Plasma samples for viral genotyping were collected at baseline, between months 1 and 3 of treatment and between months 3 and 6 of treatment. Viral RNA was extracted from plasma. A gag-protease region of 323 bp, including the end of NC, p1/TFP and p6/p6pol, was amplified by nested RT–PCR with forward primer 5'-TAAGTGTTTCAATTGTGGCAAAGAAGGGCA-3' and reverse primer 5'-TACTGATCATCTGCTCCTATC-3' in the first round, and forward primer 5'-GCCAGAAATTGCAGGGCCCCTAGG-3' and reverse primer 5'-TCCTTTAGTTGCCCCCTATC-3' in the second round. Differences in amino acid sequence with respect to the wild-type virus HXB2 were noted. Darunavir mutations were defined according to the International AIDS Society USA (IAS-USA) guidelines.

**Statistical methods**

Descriptive statistics were used to summarize the characteristics of the patients. Fisher’s exact test was used to test for significant associations between the mutation A431V in the gag sequence and two mutations in the protease sequence (L76V and M46IL). Logistic regression was used to assess whether gag mutations were predictive of the occurrence of one or more darunavir resistance mutations.

**Results**

Forty-eight PI-experienced patients displaying treatment failure on a darunavir-containing regimen were studied; genotypic
**gag mutations and darunavir failure**

Table 1. Genetic factors in the protease and gag region involved in selection of darunavir resistance mutations

<table>
<thead>
<tr>
<th>Variables</th>
<th>$P$ value</th>
<th>Odds ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Univariate analysis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insertions and/or deletions</td>
<td>0.86</td>
<td>1.1</td>
</tr>
<tr>
<td>gag reading frame</td>
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<td></td>
</tr>
<tr>
<td>mutation A431$^a$</td>
<td>0.77</td>
<td>0.84</td>
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<tr>
<td>mutation A431V$^a$</td>
<td>0.6</td>
<td>0.73</td>
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<tr>
<td>mutation K436</td>
<td>0.77</td>
<td>1.31</td>
</tr>
<tr>
<td>mutation I437$^a$</td>
<td>0.057</td>
<td>0.26</td>
</tr>
<tr>
<td>mutation I437T/V$^a$</td>
<td>0.024</td>
<td>0.19</td>
</tr>
<tr>
<td>mutation Y441</td>
<td>0.78</td>
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<tr>
<td>gag-pol reading frame</td>
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<tr>
<td>mutation D437</td>
<td>0.66</td>
<td>1.59</td>
</tr>
<tr>
<td>mutation D437N</td>
<td>0.052</td>
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<tr>
<td>mutation L441</td>
<td>0.76</td>
<td>1.56</td>
</tr>
<tr>
<td>mutation L441P$^a$</td>
<td>0.67</td>
<td>0.72</td>
</tr>
<tr>
<td><strong>Multivariate analysis</strong></td>
<td></td>
<td></td>
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<tr>
<td>Mutation I437T/V$^a$</td>
<td>0.055</td>
<td>0.195</td>
</tr>
<tr>
<td>Mutation L76V$^a$</td>
<td>0.008</td>
<td>0.049</td>
</tr>
</tbody>
</table>

$^a$Factors associated with a decrease in the risk of selection of darunavir resistance mutations.

The analysis of protease and gag was used to investigate the mutations selected by darunavir in the gag region (comprising NC-p1/TFP-p6/p6pol). The nucleotide changes leading to amino acid changes in both translational reading frames (gag and gag-pol) were explored (Figure 1). There were no genotypic changes in the gag region studied (point mutations or insertions and/or deletions) between baseline and the latest available rebound isolates from the subjects. At the latest available rebound, 87% of patients with the L76V mutation also carried the mutation A431V in the gag sequence (already present at baseline): suggesting an association between both mutations ($P = 0.05$). Logistic regression models were generated to identify if mutations associated with the risk of occurrence of at least one darunavir mutation (Table 1). Insertions and/or deletions in gag did not seem to be associated with an increased risk of selection of darunavir mutations. However, the I437T/V mutation in the gag reading frame protected against the selection of at least one darunavir mutation ($P = 0.024$; OR = 0.19). Conversely, mutation D437N, at the same amino acid position but in the gag-pol reading frame, was associated with the risk of occurrence of at least one darunavir mutation ($P = 0.052$). Multivariate analysis retained only the L76V mutation in the model, as previously reported, although the I437T/V mutation was very close to being independently associated with the risk of occurrence of darunavir mutations ($P = 0.055$).

**Discussion**

The HIV-1 protease is responsible for its own maturation from gag-pol and for processing viral polyproteins to produce mature structural and functional proteins. Assembly and maturation of the polyproteins follow a precise programme in the life cycle of HIV-1. Premature activation of the protease or partial inhibition of its activity during virus maturation leads to defects in viral assembly and consequently to the formation of aberrant non-infectious particles. We report an investigation of the impact of a mutation in the gag region NC-p1/TFP-p6/p6pol in 48 PI-experienced patients experiencing treatment failure on a darunavir-containing regimen. Two mutations were associated with a lower rate of selection of darunavir resistance mutations: I437T/V in gag and L76V in protease.

The I437T/V mutation in the gag reading frame was associated with less frequent selection of darunavir resistance mutations. Selection of amino acid changes near the NC/p1 CS resulting in PI resistance has been reported in the absence of any protease mutations. Indeed, phenotypic and genotypic resistance testing of clinical samples that lacked primary protease mutations$^{11}$ indicated that the I437V mutation is significantly associated with a more than 5-fold increase in resistance to at least one clinically used PI. The study of Nijhuis et al.$^9$ identified the I437T/V mutation in gag in vitro that increased PI resistance without protease mutations. These results have been confirmed in vivo in isolates with gag mutations (at positions 431, 436 and 437) and which did or did not carry known resistance mutations in the protease.$^9$ Moreover, in vitro selection experiments using darunavir demonstrated phenotypic resistance that could not be explained by resistance mutation in the viral protease sequence but could be explained by mutation interactions, including a CS mutation at codon 437.$^3,12$ Increased polyprotein processing due to mutations in the natural substrate rather than the enzyme itself may be a novel mechanism by which HIV develops resistance to PIs.$^9$ Indeed, particular changes at the P1 position of this CS and changes at position I437T/V were directly associated with enhanced polyprotein processing; this enhanced processing of the NC/p1 CS correlated with virological failure during PI therapy.$^9$

Consequently, viruses with the I437T/V mutation probably have increased protease efficiency due to a mutated substrate and seem not to require selection of darunavir resistance mutations in the protease gene to be resistant to darunavir.

The association between the presence of L76V at baseline and a lower risk of accumulating darunavir resistance mutations may be due to the strong effect of this mutation on darunavir resistance, consistent with the selection of other mutations not being required for darunavir-containing regimens to fail. Interestingly, we confirmed an association between the mutation L76V in the protease sequence and the mutation A431V in the gag sequence. This type of association has previously been described in lopinavir-naive patients for whom resistance produced by the L76V mutation was increased by the presence of M46I and A431V mutations in the gag CS.$^{13}$ These various observations suggest that mutation A431V in gag may influence the selection of mutation L76V in protease.

In conclusion, mutations in the gag region NC-p1/TFP-p6/p6pol may influence the selection of darunavir resistance mutations, and the I437T/V gag mutation that confers resistance to PI is particularly implicated. These results are based on genotyping and could therefore be confirmed by evaluating phenotype resistance to darunavir and replicative capacity of viruses harbouring the I437T/V gag mutations by site-directed mutagenesis. Further studies are required to determine to what extent CS mutation may explain virological failure during darunavir therapy; this would be valuable for drug development and for monitoring patients.
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Transparency declarations
None to declare.

References


