Frequency and patterns of protease gene resistance mutations in HIV-infected patients treated with lopinavir/ritonavir as their first protease inhibitor

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Background: Selection of protease mutations on antiretroviral therapy (ART) including a ritonavir-boosted protease inhibitor (PI) has been reported infrequently. Scarce data exist from long-term cohorts on resistance incidence or mutational patterns emerging to different PIs.

Methods: We studied UK patients receiving lopinavir/ritonavir as their first PI, either while naive to ART or having previously received non-PI-based ART. Virological failure was defined as viral load ≥400 copies/mL after previous suppression <400 copies/mL, or failure to achieve <400 copies/mL during the first 6 months. pol sequences whilst failing lopinavir or within 30 days after stopping were analysed. Major and minor mutations (IAS-USA 2008—after exclusion of polymorphisms) were considered. Predicted susceptibility was determined using the Stanford HIVdb algorithm.

Results: Three thousand and fifty-six patients were followed for a median (IQR) of 14 (6–30) months, of whom 811 (27%) experienced virological failure. Of these, resistance test results were available on 291 (36%). One or more protease mutations were detected in 32 (11%) patients; the most frequent were I54V (n=12), M46I (n=11), V82A (n=7) and L76V (n=3). No association with viral subtype was evident. Many patients retained virus predicted to be susceptible to lopinavir (14, 44%), tipranavir (26, 81%) and darunavir (27, 84%).

Conclusions: This study reflects the experience of patients in routine care. Selection of protease gene mutations by lopinavir/ritonavir occurred at a much higher rate than in clinical trials. The mutations observed showed only partial overlap with those previously identified by structural chemistry models, serial cell culture passage and genotype–phenotype analyses. There remained a low degree of predicted cross-resistance to other widely used PIs.

Keywords: antiretroviral, genotypic, genotype

Introduction

Lopinavir co-formulated with ritonavir has been an extensively used protease inhibitor (PI) against HIV. It is recommended for use in both first-line (developed countries) and second-line (developed and developing countries) antiretroviral therapy (ART) globally. In the UK, it has mainly been used as second-line ART or in patients with transmitted drug resistance, as

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current guidelines recommend that first-line ART should include a non-nucleoside reverse transcriptase inhibitor (NNRTI) where possible. Development of protease resistance can reduce the treatment options available and may also lead to development of resistance to other classes within the ART combination if the PI is not working effectively. Identifying predictors of protease resistance might help our ability to sequence effective ART regimens in patients with both primary (acquired) and secondary viral resistance (developed after virological failure).

A cardinal feature of randomized trials of first-line lopinavir/ritonavir-containing regimens has been the absence of major protease resistance mutations among patients who experienced virological failure3–9 and case series reports are also few in number.10,11 The recent increase in the prevalence of certain protease mutations described in unlinked resistance databases12–14 is puzzling in the light of the high genetic barrier of lopinavir/ritonavir in particular and ritonavir-boostered PIs in general.12,14,15 Our aim was to describe the frequency and patterns of protease resistance among UK patients who experience virological failure whilst taking lopinavir/ritonavir as their first PI.

Methods

The UK Collaborative HIV Cohort (CHIC) study collates routine data on HIV-positive individuals attending some of the largest clinical centres in the UK. It includes all individuals aged ≥16 years who have attended one or more of the collaborating centres at any time since 1996. Electronic downloads of specified data are obtained annually from each centre. pol gene sequences from patients enrolled in UK CHIC are obtained via linkage to the UK HIV Drug Resistance Database, which collates the vast majority of genotypic resistance tests conducted in the UK. Both studies have UK Multicentre Ethics Committee approval (MREC/00/7/47 and MREC/01/12/10), which, because data are anonymized, did not require individual patient consent. Eligible patients had received lopinavir/ritonavir as their first PI, either while naive to ART or having previously received non-PI-based ART. Data were not available on dosing schedules of lopinavir/ritonavir, although it is likely that the majority received it twice daily. We reviewed the clinical notes of patients fulfilling the inclusion criteria for this analysis in whom protease mutations were detected (definitions below), to exclude the prescription of other PIs not recorded in local electronic databases. In addition, a clinical virologist (D. P.) reviewed mutational patterns in reverse transcriptase: if a major IAS mutation was detected in the absence of drug exposure that selects for that mutation it was assumed that the recorded antiretroviral history was incomplete and such patients were excluded from both numerator and denominator.

Virological failure was defined as either viral load >400 copies/mL after previous suppression to <400 copies/mL, or failure to achieve viral suppression <400 copies/mL during the first 6 months of therapy. Patients were censored at the time of starting any PI other than lopinavir/ritonavir, discontinuation of lopinavir/ritonavir, or their last viral load measurement; whichever occurred first. The analysis of protease resistance was based on resistance tests performed while the patient was receiving, or within 30 days after stopping, lopinavir/ritonavir. We considered all protease mutations, both major and minor and lopinavir-associated and non-lopinavir-associated, according to IAS-USA 2008.16 However, some of these mutations are also natural polymorphisms and their acquisition in an individual patient cannot be unequivocally established in the absence of a baseline test, which was generally lacking in this study population. We therefore first examined the prevalence of each mutation among 18791 ART-naive patients in the UK and excluded positions where the prevalence of mutational variants exceeded 1%; this applied to positions 10, 13, 16, 20, 36, 60, 62, 63, 64, 69, 71, 77 and 93. In addition, V82I (prevalence 5.3%) was excluded, although all other mutations at this position were considered. It is noted that patients with these variants were retained in the analyses of other positions. The mutations included in the final analysis were as follows: L24I, D30N, V32I, L33F, E34Q, E35G, K43T, M46I/L, I47V/A, G48V, 150V, F53L/Y, L54F/I/A/M/MTS, Q58E, G73C/S/T/A, T74P, L76V, V82A/F/T/S/L, N83D, I84V, 185V, N88D/S, L89V and L90M. For each mutation that was observed at virological failure we applied Bayes' theorem17 to estimate the probability that the mutation was acquired as opposed to transmitted. For this calculation we made the simplifying assumption that transmitted mutations persist indefinitely; this assumption is conservative in that it produces an under-estimate of the probability that the mutation was acquired.

Predicted susceptibility to lopinavir, tipranavir and darunavir was determined using the Stanford HIVdb algorithm version 6.0.10.18 Subtype was inferred from pol sequences using the REGA HIV subtyping algorithm.19 For each patient, the duration of lopinavir/ritonavir exposure and the area under the viraemia curve (AUC) were calculated (as duration of exposure in years multiplied by HIV RNA log10) from initiation of lopinavir/ritonavir to the first sample with protease mutations, or to the last sample that was genotyped if protease mutations were never detected. Viral loads were not included for patients in whom a treatment interruption was recorded. Undetectable viral load values were imputed at the lower limit of detection for the assay. Logistic regression analysis was used to examine the relationship between the presence of protease mutations with viral load closest to the resistance test sample, duration of lopinavir/ritonavir exposure and AUC.

Results

A total of 3056 patients started lopinavir/ritonavir as their first PI. One thousand four hundred and three (46%) were homo-/bisexual males, 449 (15%) were heterosexual males, 821 (27%) were heterosexual females and 383 (13%) were another/unknown risk group. The majority of patients were white (1574, 52%), 1113 (36%) were black and 369 (12%) were other/unknown ethnicity. Around half (1580, 52%) were naive to ART (and had thus started lopinavir/ritonavir combination ART as their first-line regimen), 569 (19%) had received previous NNRTI-based ART only and the remaining 907 (30%) had previously taken other regimens. Most patients (2627, 86%) commenced lopinavir/ritonavir in combination with NRTIs; of the remainder, 269 (9%) received lopinavir/ritonavir with both NRTIs and an NNRTI, 102 (3%) received lopinavir/ritonavir monotherapy and 58 (2%) received lopinavir/ritonavir with an entry inhibitor, an integrase inhibitor or an NNRTI only.

Eight hundred and eleven patients (27%) experienced virological failure over a median (IQR) follow-up of 14 (6–30) months, with a similar failure rate if lopinavir/ritonavir was used as the first line or after a non-PI-based regimen (P=0.07, log-rank test) (Figure 1). Among patients who experienced virological failure, 291 (36%) had a total of 427 resistance tests (range 1–6 per patient) while receiving lopinavir/ritonavir (94% of tests) or within 30 days of stopping the drug (6% of tests). Among the 291 patients who were genotyped, 130 (45%) were infected with a subtype B virus, 59 (20%) with subtype C, and
other subtypes appeared in lower proportions (A 11%, AG 8%, other 9%, unclassified 7%).

Thirty-seven patients were initially identified with protease mutations but five were excluded because of suspicion that their antiretroviral history was incomplete or that data linkage errors had occurred, leaving 32 of 286 (11%) patients who developed resistance. The individual protease mutations detected at the first resistance test are listed in Table 1, the most common of which were I54V (n=12) and M46I (n=11). Of the major lopinavir mutations, V82A, I47V or I47A, and V32I were observed in seven, three and two patients, respectively. Four mutations (Q58E, T74P, I85V and L89V) are not considered to be lopinavir-associated, although two of these (T74P and L89V) were observed in a single patient only. It is noted that only 9 of the 32 patients had a resistance test preceding their exposure to lopinavir/ritonavir, 10 showed no evidence of a positive association between I54V and V82T (not shown) revealed no significant effects apart from weak evidence of a positive association between I54V and V82T (P=0.07, Fisher’s exact test), although the power to detect such associations was small. In 16 (50%) patients the protease mutation(s) were found without any major reverse transcriptase mutation. There was no difference in the viral subtype distribution between patients who failed virologically with and without protease resistance (P=0.23, Fisher’s exact test), i.e. there was no evidence that certain subtypes are predisposed to developing lopinavir resistance. The HIV RNA value at or closest to the date of the resistance test ranged widely between 170 and 304,000 copies/mL, with non-adherence a possible cause of some of the higher values. The predicted level of viral resistance to lopinavir and cross-resistance to tipranavir and darunavir is also shown. The number of samples classified either as ‘susceptible’ or ‘potential low-level resistance’ was 14 (44%) for lopinavir, 26 (81%) for tipranavir and 27 (84%) for darunavir.

There were trends towards a higher likelihood of protease mutations with longer exposure to lopinavir/ritonavir (OR 1.22 per year, 95% CI 0.95–1.57, P=0.13) and with higher viral AUC (OR 1.09 per log_{10} copies/mL-years, 95% CI 1.00–1.20, P=0.06). Mean viral load measured on the genotyped sample (or closest sample if not available) was similar in patients with and without protease mutations (3.82 log_{10} copies/mL versus 4.06 log_{10} copies/mL; P=0.22, t-test).

**Discussion**

Randomized controlled trials of first-line lopinavir/ritonavir-based combination regimens have generally failed to identify protease gene mutations associated with virological failure.3–5,8,9 More information on the mechanisms of viral escape from lopinavir/ritonavir resistance is still required.

**Table 1. Observed protease mutations and their prevalence among ART-naive patients**

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Number (%) in patients failing lopinavir-based therapy</th>
<th>Prevalence (%) in ART-naive patients</th>
<th>Probability (%) that mutation was acquired rather than transmitted</th>
</tr>
</thead>
<tbody>
<tr>
<td>V32I</td>
<td>2 (0.7)</td>
<td>0.037</td>
<td>94.6</td>
</tr>
<tr>
<td>L33F</td>
<td>1 (0.3)</td>
<td>0.346</td>
<td>100.0</td>
</tr>
<tr>
<td>M46I</td>
<td>11 (3.8)</td>
<td>0.256</td>
<td>94.4</td>
</tr>
<tr>
<td>M46I/L</td>
<td>1 (0.3)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>I47V</td>
<td>1 (0.3)</td>
<td>0.048</td>
<td>86.0</td>
</tr>
<tr>
<td>I47A</td>
<td>1 (0.3)</td>
<td>0.005</td>
<td>98.5</td>
</tr>
<tr>
<td>I47A/V</td>
<td>1 (0.3)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>F53L</td>
<td>2 (0.7)</td>
<td>0.139</td>
<td>79.8</td>
</tr>
<tr>
<td>IS4V</td>
<td>12 (4.2)</td>
<td>0.160</td>
<td>97.1</td>
</tr>
<tr>
<td>(Q58E)</td>
<td>3 (1.0)</td>
<td>0.421</td>
<td>72.8</td>
</tr>
<tr>
<td>(T74P)</td>
<td>1 (0.3)</td>
<td>0.037</td>
<td>100.0</td>
</tr>
<tr>
<td>L76V</td>
<td>3 (1.0)</td>
<td>0.037</td>
<td>96.4</td>
</tr>
<tr>
<td>V82A</td>
<td>7 (2.4)</td>
<td>0.021</td>
<td>99.1</td>
</tr>
<tr>
<td>184V</td>
<td>3 (1.0)</td>
<td>0.096</td>
<td>96.9</td>
</tr>
<tr>
<td>(185V)</td>
<td>2 (0.7)</td>
<td>0.250</td>
<td>63.6</td>
</tr>
<tr>
<td>(L89V)</td>
<td>1 (0.3)</td>
<td>0.059</td>
<td>82.8</td>
</tr>
<tr>
<td>L90M</td>
<td>2 (0.7)</td>
<td>0.570</td>
<td>58.5</td>
</tr>
</tbody>
</table>

Mutations in bold are major lopinavir mutations by IAS 2008. Mutations in parentheses are not lopinavir-associated by IAS 2008.

*Percentage relative to total number of patients with one or more resistance tests (n=291).

Prevalence in ART-naive patients based on 18774–18791 tests.

*Estimated using Bayes' theorem (see Methods section).
ritonavir has emerged from analyses of clinical trials and monotherapy studies, such as the MONARK trial, which described major protease resistance mutations in 5 of 83 patients treated for up to 96 weeks, with the following patterns: L76V (n = 2), M46I, L10F + V82A and M46I + L76V. In the context of combination therapy, we are aware of only a total of six reports of lopinavir/ritonavir resistance from three cases series.

The lack of resistance identified in clinical trials may be due to the relatively short follow-up and a tendency to switch therapy in the face of low-level viraemia (participants are more closely monitored) and consequently minimal drug selection pressure. This highlights the importance of studying ‘real-world’ patient cohorts in the setting of routine clinical care, where there is typically much more prolonged drug selection pressure and a spectrum of co-administered therapies. Our analysis identified a total of 32 cases in which protease gene mutations were apparently due to lopinavir/ritonavir exposure. The three most common substitutions observed were M46I, L10F and V82A; interestingly, the L76V mutation was observed in only 3 of 32 cases. However, it is worth noting that some of the mutations, such as those at positions 46 and 82, may have been selected by low-dose ritonavir rather than by lopinavir.

Interestingly, the mutations that were selected in our study showed only a partial overlap with mutations identified by structural chemistry models, serial passage in cell culture, genotype-phenotype associations and trend analyses of resistance databases. For example, the T91S mutation was not observed despite its appearance in two independent serial passage experiments. Second, there was only a single case of I47A despite a clear temporal relationship between its appearance and the availability of lopinavir/ritonavir in the large QUEST resistance database. Third, the K20M/R mutation did not occur despite the fact that this mutation results in >20-fold reduced susceptibility to lopinavir/ritonavir in clinical viral isolates. Fourth, although the I84A and I84C mutations confer a greater reduction in lopinavir susceptibility than the I84V mutation in vitro, only the latter was observed in our study. Finally, to our knowledge this is the first description of the association between lopinavir/ritonavir and the T74P, I85V and L89V mutations. We note that Q58E has been described in lopinavir/ritonavir failures previously.

Our study has several limitations. First, our dataset did not include gag gene sequences matched to protease sequences. Recent research indicates that mutations in this gene may reduce susceptibility to PIs either alone in the context of wild-type protease or by facilitating the growth of virus with highly resistant viral protease. This could partly explain why virological failure in the context of ritonavir-boosted PIs in general, and lopinavir/ritonavir in particular, frequently occurs without any apparent protease resistance. Second, only approximately one-third of patients with virological failure had their virus sequenced. Lacking information on the reasons why some patients underwent testing while others did not, it is impossible to rule out the possibility of testing selection bias. This could have operated in either direction, i.e. causing the true frequency of resistance to have been under-estimated or over-estimated. Third, as most patients in whom protease resistance mutations were identified lacked a baseline resistance test, the evidence that the mutations were caused by lopinavir/ritonavir exposure is only indirect.

Finally, the association between resistance and total viraemia (AUC) suggests that accumulation of protease mutations is more likely with prolonged virological failure. However, a reassuring finding for clinical practice was the preserved predicted susceptibility to two PIs (darunavir and tipranavir) thought to be suitable for use after previous PI failure.

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Disclaimer
The views in the publication are those of the authors and not necessarily those of the Medical Research Council.

Supplementary data
Table S1 is available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).

References


