Differentiation of genotypic resistance profiles for amprenavir and lopinavir, a valuable aid for choice of therapy in protease inhibitor-experienced HIV-1-infected subjects

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Keywords: amprenavir, lopinavir, genotype, resistance, HIV

Introduction

One of the major challenges to the successful long-term treatment of HIV-1 infection is overcoming the development of increasing levels of antiretroviral resistance that often accompany the failure of successive treatment regimens. In order to meet this challenge a good understanding of genotypic resistance profiles and the potential for cross-resistance within each class of antiretrovirals is essential. For the protease inhibitors (PIs), cross-resistance is complex, as a result of the large number of mutations involved. Amprenavir and lopinavir are potent PIs used in ritonavir-boosted regimens, often in patients who have already experienced treatment with other PIs. The resistance profiles of these two PIs overlap to a certain extent but also contain some important differences that can be exploited in the choice of optimal treatment for PI-experienced patients. This article reviews our own research and that of others, in order to clarify the similarities and the differences between the genotypic resistance profiles for amprenavir and lopinavir. Whereas phenotypic data are valuable for understanding resistance, HIV-1 genotyping is critical in making the optimal choice between these two drugs in PI-experienced subjects.

In vitro and in vivo resistance profiles for amprenavir and lopinavir

The key amino acid substitution selected during several in vitro passage experiments with amprenavir is I50V, located in the active site of the protease and usually accompanied by an M46I/L and, less frequently, by an I47V substitution.1,2 Analysis of virus isolates from PI-naive patients treated with unboosted amprenavir has identified four resistance pathways involving the substitutions I50V, M46L/M, V32I + I47V and gag cleavage site mutations or, less frequently, the I50V substitution has been associated with the highest amprenavir trough plasma concentrations (Cmin) whereas I54L/M, which confers the lowest levels of amprenavir resistance and has the least impact on fitness, is associated with the lowest Cmin.5,6 In a Phase III trial of once-daily ritonavir-boosted GW433908 (a prodrug of amprenavir) in antiretroviral-naive subjects, plasma levels of amprenavir are elevated to the extent that no PI resistance-associated mutations have been observed after 48 weeks of treatment.8

In vitro virus passage experiments using lopinavir (with or without ritonavir) have primarily selected either the I84V mutation in combination with L10F, M46I, T91S, V32I, I47V and gag cleavage site mutations or, less frequently, the I50V substitution has been associated with the highest levels of amprenavir resistance and has the least impact on fitness, is associated with the lowest Cmin.5,6 Clones with a V82A substitution have also been observed.9 The high plasma levels of lopinavir achieved in subjects treated with lopinavir/ritonavir mean that virological failure in antiretroviral-naive patients has been rarely accompanied by genotypic changes in the protease. Where genotypic changes were seen, these were secondary mutations.10 In PI-experienced patients, lopinavir/ritonavir treatment selects various different genotypic changes on the background of existing PI mutations, most often L10F, M46I, IS4V and V82A and occasionally I50V.11

The impact of specific mutations on cross-resistance and response to amprenavir and lopinavir in PI-experienced subjects

Studies of viruses from PI-experienced patients indicate that the degree of phenotypic cross-resistance to both amprenavir and lopinavir is considerably less than to other PIs including atazanavir and saquinavir.13–16 Nevertheless, increasing prior PI experience13–17 and prior treatment with ritonavir, nelfinavir or indinavir16,17 leads to an increased likelihood of resistance to both these agents, indicating some overlap between the resistance profiles of these PIs. Differ-
Table 1. Comparison of frequencies of mutations significantly ($P < 0.001$) associated with reduced susceptibility to amprenavir or lopinavir in either of two different data sets

<table>
<thead>
<tr>
<th>Data set</th>
<th>Mutation</th>
<th>n (%)</th>
<th>resistance cut-off$^a$ for amprenavir</th>
<th>resistance cut-off$^a$ for lopinavir</th>
<th>resistance cut-off$^b$ for amprenavir</th>
<th>resistance cut-off$^b$ for lopinavir</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>≥2.5</td>
<td>≥5</td>
<td>≥2.5</td>
<td>≥10</td>
</tr>
<tr>
<td>Cohort 1</td>
<td>Total</td>
<td>271</td>
<td>92</td>
<td>46</td>
<td>164</td>
<td>77</td>
</tr>
<tr>
<td>Cohort 2</td>
<td></td>
<td>233</td>
<td>158</td>
<td>58</td>
<td>175</td>
<td>75</td>
</tr>
</tbody>
</table>

$^a$Fold change in drug susceptibility above which samples were classified as resistant for the purposes of the analysis. Two different cut-offs were used for each drug. A 2.5-fold reduction in susceptibility represented the biological cut-off for both drugs. The lopinavir/ritonavir clinical cut-off, described in the Kaletra package insert, of a 10-fold reduction in susceptibility was used for lopinavir. Since no clinical cut-off has been determined for amprenavir, a five-fold reduction in susceptibility was chosen on the basis of a minimum two-fold increase in plasma amprenavir levels in patients treated with the ritonavir-boosted drug.$^b$

$^b$Cohort 1 comprised 271 longitudinal plasma samples from 207 PI-experienced subjects enrolled as study participants in the Pacific Oaks Clinic Population Study at the Pacific Oaks Clinic (Beverly Hills,
ences between the resistance profiles of lopinavir and amprenavir are suggested by an association of prior saquinavir exposure with reduced susceptibility to lopinavir but not to amprenavir.16

We have analysed two large sets of matched genotypic and phenotypic data in order to determine the genotypic correlates of cross-resistance to amprenavir and lopinavir in subjects previously treated with other PIs. The first data set comprised 271 plasma samples from a cohort of 207 HIV-1-infected subjects enrolled in the Pacific Oaks Clinic Population Study.18 These subjects had received one to four consecutive or concurrent non-boosted PIs for at least 4 months and had either failed to attain a virological response (defined as HIV-1 RNA < 400 copies/mL) or had attained a virological response and subsequently experienced virological rebound (defined as HIV-1 RNA > 1000 copies/mL after virological response). PIs in the regimens for these subjects included indinavir, ritonavir, saquinavir, amprenavir and nelfinavir. The second data set included a random selection of 233 samples from the VIRCO database.18 Samples were derived from PI-experienced patients but no detailed treatment history was available. Univariate analyses of these data sets identified several differences between the profiles of mutations significantly associated with two levels of reduced susceptibility to each drug (≥2.5-fold and ≥5-fold for amprenavir and ≥2.5-fold and ≥10-fold for lopinavir, Table 1). In both data sets, and at both levels of resistance, more mutations were associated with lopinavir resistance than with amprenavir resistance. The substitutions I54T or V and V82A were strongly associated with lopinavir resistance but were not associated with amprenavir resistance. In contrast, the relative incidence of M46I and I84V was higher in the amprenavir-resistant virus populations than in lopinavir-resistant viruses. Mutations L10I, M36I, M46I, G73S and L90M were associated with resistance to both drugs in at least one of the cohorts studied. D30N was associated with susceptibility to amprenavir and lopinavir in both data sets and V77I was significantly associated with susceptibility to lopinavir in one of the data sets. In several cases, discordance in the results between the two studies may have been because of a low incidence of the mutation in one or both data sets (e.g. L24I, L33F, G48V, F53L, I54T) or to low relative incidence values for mutations with a high incidence (e.g. L10I, G73S, L90M).

In isolates with multiple mutations, a univariate analysis may over-estimate the contribution of certain mutations to drug resistance because of complex linkage patterns. Therefore, a multivariate analysis was carried out on one of the data sets (Cohort 1, the Pacific Oaks Clinic population) including mutations L10I, M46I, I84V and L90M as covariates for amprenavir resistance (≥2.5-fold) and mutations L10I, G48V, I54V, I54T and V82A as covariates for lopinavir resistance (≥10-fold). The presence of I84V was most strongly associated with amprenavir resistance (P < 0.0001).16 L90M was found along with I84V in 65% of amprenavir-resistant isolates but was not independently associated with resistance. For lopinavir, the multivariate analysis revealed L10I and I54V as most strongly associated with a ≥10-fold reduction in susceptibility (P < 0.0002). The G48V mutation was commonly associated with V82A and 79% (23/29) of isolates carrying these two mutations were resistant to lopinavir. Isolates carrying G48V in the absence of V82A displayed intermediate levels of resistance (≥3.5-fold, <10-fold).

Other studies have generally confirmed these observations identifying the I84V as a key determinant of reduced phenotypic susceptibility to amprenavir14,15,19 as well as some associations between amprenavir resistance and M46I/L and L90M.14,19 Univariate analysis from two different studies also reported a significant association between amprenavir resistance and mutations at codon 54, but did not differentiate between different amino acid substitutions (L, M or V).14,19 In our analysis I54V, the most commonly seen substitution at this position in PI-experienced patients, was not associated with amprenavir resistance. In vitro site-directed mutagenesis studies also show that the I54V has no affect on amprenavir susceptibility.3 In addition, treatment of PI-experienced patients with amprenavir during the CNA2007 study resulted in the replacement of 54V or I with either 54M, wild-type, or 54L in several cases.4

In a study carried out by researchers from VIRCO on 1300 samples derived from PI-experienced subjects, the mutations (order of decreasing prevalence) L10I, A71V, V82A, L90M, I54V, M46I, I84V, G73S and K20R were more frequent in viruses demonstrating resistance to lopinavir (≥10-fold).20 Researchers at Abbott Laboratories analysed the genotypic correlates of reduced phenotypic susceptibility to lopinavir in 112 clinical isolates from PI-experienced patients and identified 11 amino acid substitutions that appeared to be involved in lopinavir resistance (L10F/R/V, K20M/R, L24I, M46I/L, F53L, I54L/T/V, L63P, A71I/L/T/V, V82A/F/T, I84V and L90M).21 The number of substitutions from this list (referred to as the ‘lopinavir mutation score’) correlated with the reduction in susceptibility to lopinavir (R² = 0.62) and a score of six or more gave a mean reduction in susceptibility of ≥10-fold. Kempf et al. have also applied the mutation score approach to correlate genotype with virological response.22 They examined the virological response of 50 multiple PI-experienced HIV-infected subjects over 72 weeks of treatment with lopinavir/ritonavir plus efavirenz and nucleoside reverse transcriptase inhibitors (study M98-957). A virological response to <400 HIV-1 RNA copies/mL was observed in 91% (21/23), 71% (15/21) and 33% (2/6) of subjects with baseline lopinavir mutation scores of 0–5, 6–7 and 8+, respectively. Several studies carried out on PI-experienced patients treated with lopinavir/ritonavir as part of expanded access programmes have also aimed to identify mutations associated with reduced virological response to lopinavir. In the Canadian expanded access programme, a study of 167 PI-experienced patients treated with lopinavir/ritonavir identified L90M as an independent predictor of reduced response.23 However, this study only considered mutations included in the Abbott lopinavir mutation score. In a study of 700 patients in the French Drug Agency Temporary Authorisation
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for Use (ATU) a univariate analysis identified mutations at positions 54, 82, 10 and 46 as being statistically significantly (P < 0.01) associated with virological failure. In a stepwise logistic regression, mutations at the same four positions were determined to be independently associated with virological failure. The specific amino acid substitutions seen in this study were not given. A second ATU study, involving 68 patients, found that M46I, I45V and V82A were associated with a poor response (P < 0.25) in a univariate analysis. A multivariate analysis identified I54V and a lopinavir mutation score of greater than five as being independent predictors of virological failure. A high number of previous PI, prior therapy with ritonavir or indinavir, absence of co-prescription of efavirenz, and a lower exposure to lopinavir or lower lopinavir trough concentrations were also independently correlated with poor response. Further analysis of the ATU data has led to the definition of an alternative mutation score that includes mutations at positions 10, 20, 24, 33, 36, 47, 48, 54, 82 and 84 (specific substitutions not specified). A stepwise logistic regression analysis of the ATU data and data from study M98-957 identified the ATU mutation score as being a better predictor of virologic response than the original lopinavir mutation score. The Clinic-Based Investigators Group also examined genotypic predictors of clinical response to lopinavir in a multicentre clinical cohort (n = 77), and found that mutations 20M, 36I, 46L, 54S/V, 71V, 73S and 82A were significant predictors of response (P < 0.05), with the specific mutations 46L, 71V and 82A being the strongest predictors. A weighted genotype score appeared to predict response better than summing mutations, although both the Abbott lopinavir mutation score and the ATU set were also tested and both were predictive of virological response over 24 weeks. Other unusual amino acid variants at resistance associated sites may also impact lopinavir response, as recent presentations have identified the less common V84A and V84C variants, and the I47A variant as increasing resistance to lopinavir.

Conclusions

Based on the above analyses, we believe that genotyping will be important to aid the optimal choice of the next PI to use for patients failing on a PI-containing regimen. Although a number of protease mutations clearly impact on the levels of susceptibility seen for both amprenavir and lopinavir, particularly when ≥5 appear together, differences in the resistance profiles of these two drugs do exist suggesting that each drug will be of value in the treatment of different subjects. In particular, different mutations at codon 54 affect the two drugs differently; I54V being more critical for lopinavir resistance and I54L/M for amprenavir resistance. Furthermore, I54V has been independently associated with failure in PI-experienced patients on lopinavir/ritonavir. This highlights the importance of considering specific amino acid substitutions in mutation scores used to predict response, rather than considering any change at a particular amino acid position. The V82A mutation is clearly involved in lopinavir resistance and does not have any significant impact on amprenavir resistance, whereas the I84V mutation appears to affect resistance to amprenavir more than to lopinavir. In treatment-naive subjects, no key PI resistance mutations have been seen to emerge during virological failure on either lopinavir/ritonavir or the once-daily regimen of GW433908/ritonavir. Therefore, boosted PI regimens should increase the potential for successful treatment outcomes and genotypic interpretation systems may need to be adapted accordingly.

Acknowledgements

We gratefully acknowledge the continued support of the GSK statistical team, Qiming Liao, Allison Florance and Naomii Richards. We wish to thank Esther Race for her technical writing assistance.

References


Cross-resistance between amprenavir and lopinavir

Amprenavir-resistant viruses generated in vitro (by passage or mutagenesis) carrying the combination of H10I, M46I, I47V and I50V display greatly reduced susceptibility (>10-fold change in IC50) to lopinavir and (unpublished data generated by GSK). However, viruses selected by first-line amprenavir treatment in vivo have displayed only minimal levels of cross-resistance with the four pathways observed (mean 2.5-fold overall). Of the key amprenavir resistance mutations (I50V, I54L/M, V32I + I47V and I84V), only the less commonly selected I84V is found in the list of lopinavir resistance mutations identified by Kempf et al. However, Parkin et al. have suggested an updated algorithm for lopinavir resistance based on their analysis of a larger and more diverse data set from 1418 virus samples derived from patients with various PI resistance profiles. They identified several mutations associated with lopinavir resistance that were not included in the Abbott lopinavir mutation score, including I50V and I54M when present with other PI mutations including some primary mutations. Further evidence for the involvement of the I50V mutation in lopinavir resistance comes from its selection during lopinavir/ritonavir treatment of PI-experienced patients. At present, I50V is relatively rare in PI-experienced subjects and although being selected by amprenavir is only one of four possible resistance pathways, all of which give limited cross-resistance to lopinavir. The impact of this mutation on future PI therapy still needs to be evaluated fully.


